ASSESSING THE HEALTH OF HARBOR SEALS IN ALASKA

A

THESIS

Presented to the Faculty

Of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

By

Stephen John Trumble, B.S., M.S.

Fairbanks, Alaska

May 2003

ASSESSING THE HEALTH OF HARBOR SEALS IN ALASKA

By

Stephen John Trumble

RECOMMENDED:

Advisory Committee Chair

Program Head

APPROVED:

Dean, School of Fisheries and Ocean Sciences

Dean of Graduate School

6-2-03

Date

ABSTRACT

Declining populations of pinnipeds in the Gulf of Alaska, possibly resulting from changes in prey quality, prompted research to determine the population health status of harbor seals (*Phoca vitulina*) using blood chemistry and digestive constraints. Blood chemistry and morphology reference range values between two harbor seal pup populations in Alaska, one population in continued decline, Prince William Sound, and another in recent increase, Tugidak Island, offered clues that blood values can vary on the population scale and that health assessment must utilize an appropriate set of reference values for valid comparisons. Subsequently, a captive study involving harbor seals yielded changes in ten blood chemistry or hematology values as a function of season and diet. These data provided evidence that populations may have distinct "identities" based on blood chemistry values. The "metabolic identity" of a population provides evidence of the relationship between environmental stressors and the genetic capacity of the animal to respond to metabolic demands. This made it possible to better understand population level differentiation in plasma chemistry values and thus assess the health of animals occupying the outlier regions of populations, since these regions are often suggestive of poor health. A captive study involving harbor seals, which are known to consume the low quality prey (pollock) implicated in the declines of many species of birds and mammals in the Gulf of Alaska, yielded consistent dry matter digestibility resulting in greater gut fill from pollock than from herring. Digestible

energy intakes from pollock were greater than from either herring or the mixed diet. Lipid digestibility of herring declined from 90% to 50% when lipid intake exceeded 60 g $kg^{-0.75}$ d^{-1}. Results of this study imply that a flexible digestive system for harbor seals can compensate for ingesting a prey of low energy density by increasing gut fill and enhancing protein and lipid assimilation, to sustain digestible energy intake. In other words, harbor seals can offset differences in prey quality if prey availability and abundance does not limit the physiological plasticity of their digestive system to maintain their supply of energy and nutrients.

TABLE OF CONTENTS

LIST OF FIGURES

Number Page

LIST OF TABLES

Number Page

ACKNOWLEDGMENTS

Son, if you really want something in this life,
you have to work for it. Now quiet! They're
about to announce the lottery numbers.

-- Homer Simpson

I wish to extend my deepest gratitude to the myriad of people who have supported me and kept me focused while undertaking a project of this scale. Specifically, I would like to thank my major professor, friend, mentor, Mike Castellini, who openly shared his wisdom and insight in matters of science and life. Also, to Maggie Castellini, the person who was there to right the ship when it began to list. To Kate Wynne, who encompasses all that is genuine in our field, thank you for your enthusiasm, hard word, drive and ability to have fun under any situation. Brian Barnes showed me that questioning brings about science and that good science just doesn't happen. Perry Barboza showed me that perseverance pays off, no matter how many edits it takes! Thank you for your humor and direction. Thanks to Paul Thompson who through his work and dedication reminded me that science is exciting and new questions can come from every project. I would also like to thank the two *de facto* members who graciously provided insight on my work.

I owe a great debt of gratitude to Dr. M. Castellini for providing the funding for all of the research between the following pages. These projects could not have been

completed without the support of the Rasmuson Fisheries Fellowship. Their generous stipend along with the insight from the Rasmuson Fisheries Board and Dr. Tyler was a great asset during my tenure at UAF. I would like to thank Dr. Bob Small from the Alaska Department of Fish and Game who became a colleague, friend and more importantly made me realize that I cheer for the right hockey team.

I want to thank my friends and family for providing emotional support and encouragement throughout this degree. I want to especially thank my brother Mark who made me realize that life is not all graphs and numbers and that nothing is as good as a Saturday spent with family. This work is dedicated to my Mom, Dad, and Brothers and Sisters; you have helped nurture my body and soul. To my mother, Sharon, you showed me that love comes in many forms, and to my sister Kristen who had science in her blood. You give me strength. To Suzanne, you came into my life like a warm breeze and helped carry me through the tough final stages of this work. I give you my heartfelt love and appreciation.

S.J. Trumble conducted all work in this study and the responsibility of co-authors in published chapters was to assist in funding and publication. Support for this work was provided by Alaska Sea Grant with funds from the National Oceanic and Atmospheric Administration Office of Sea Grant, Department of Commerce, under grant no.NA 86RG0050 (project no.R/08-08), and from the University of Alaska with funds appropriated by the state. This research was conducted with authorization from the University of Alaska Institutional Animal Care and Use Committee.

I wish to thank the following for granting me access to their knowledge, graciously offering help or keeping me sane: Dr. Jennifer Burns, Dr. Dan Costa, Dr. Nick Gales, Tamara Mau, Dion Oxman, Dr. Brian Fadely, Dr. Amy Hiron, Dr. Leslie Cornick, Dr. Lori Polasek, Dr. Markus Horning, Dr. Terrie Williams, Shawn Harper, Kelly Hastings, Kathy Frost and Lloyd Lowry, Dr. Susan Henrichs, Dr. Vera Alexander, Susan Inglis, Dr. Lorrie Rea, Brent Howard, David Cook, Bud Patterson, Rodney Martin, Laura Bender, Tania Clucas and the SFOS staff, and the numerous people who have crossed my path throughout the years.

1

INTRODUCTION

There is a growing body of research demonstrating the potential importance of using blood chemistry as an index of health or energetic status in wild animal populations. Specifically, determination of blood chemistry and hematological values that are sensitive to environmental variation permits changes in physiology to be examined (Seal et al. 1981). For example, blood constituents can reflect intake of protein and energy as well as the degree of fasting (de Swart et al. 1995, Rea et al. 1998, Thompson et al. 1997). Increasing our knowledge of seasonal metabolic patterns is essential to understanding ecological and nutritional requirements in free-ranging animals. This is a particularly important need for Alaskan pinnipeds. It has been hypothesized that nutritional stress, from a shift in prey, has contributed to the decline of many marine mammal species, including harbor seals (*Phoca vitulina richardsi*) and Steller sea lions (*Eumetopias jubatus*), over the past three decades in the Gulf of Alaska (Pitcher 1990, Small et al. 2000).

The overall goal of this thesis is to address the nutritional stress hypothesis from a physiological approach. Chapter 2 (Trumble and Castellini 2002) presents the results for differences in harbor seal pup blood chemistry and morphometrics between declining seal populations in Prince William Sound (PWS) and increasing populations on Tugidak Island. Chapter 3 examines if "clinical" determinations of blood chemistry can be used to designate individuals as outliers. Chapter 4 illustrates

how feeding trials using captive harbor seals can help establish the level of seasonal and dietary variation associated with blood chemistry, hematology and morphology. Results presented in Chapter 5 demonstrate how changes in digestive efficiency accompany changes in nutrient loads for harbor seals fed high and low quality prey (Trumble et al. 2003).

Unlike terrestrial mammals, pinnipeds offer a unique challenge when collecting and interpreting data. The harbor seal (*Phoca vitulina*; Phocidae) is one of 8 pinniped species inhabiting the Alaska coast and has population estimates between 200,000 and 300,000 (Small 2000). Although primarily marine, these relatively small phocids spend nearly half of their lives hauled out of the water on reefs, sand or gravel beaches, mud bars, or glacial and sea ice resting, giving birth, and nursing their pups (Slater and Markowitz 1983, Pitcher 1980, Brown and Mate 1983). In Alaska, single pups are born between May and mid-July and are weaned after a 4 to 6 week lactation period. Harbor seal milk is approximately 45% fat, 9% protein, and 46% water, with traces of lactose (Riedman 1990). The extremely high fat content of the milk insures a nearly doubling of weight in pups by the end of weaning, which occurs within 6 weeks. Because blood oxygen stores are not fully developed at weaning, harbor seals' diving and foraging abilities do not approach juvenile or adult levels until approximately one year of age (Burns et al. 1998). Sexual maturity in the harbor seal occurs between ages 3 and 7 years of age. Reproductively aged females show strong fidelity to birthing sites and mate shortly after the termination of lactation.

Juveniles and adults are known to be opportunistic predators whose diet varies dramatically throughout their range (Pitcher 1977, Jeffries 1986, Brown and Mate 1983, Harvey 1989). Approximately 1300 scats were collected during the 1990's along the Kodiak Archipelago and in the Bering Sea; of these identified the most frequently occurring prey was walleye pollock (*Theragra chalcogramma*; 50%) and arrowtooth flounder (*Atheresthes stomias.*; 33%). Other important prey items included Irish lord (*Hemilepidotus* sp.), sandlance (*Ammodytes sp.*), rock sole (*Lepidopsetta bilineata*), flounders (Pleuronectidae), sculpin (Cottidae), yellowfin sole (*Limanda aspera*), rainbow smelts (*Osmerus sp.*; 26%), and tomcod (*Microgadus sp.*). Preliminary results suggest regional differences in diet diversity (Jemison in Small 2000).

Absent from this list of prey items is Pacific herring (*Clupea pallasi*). During an oceanographic regime shift in the 1970's the biomass of herring declined and was replaced by the less energy dense prey pollock (Ohtani and Azumaya 1995). There is some controversy surrounding whether or not the decline in marine mammal and bird species in Alaska coincides directly with the shift to a less energy dense prey. The nutrition-based hypothesis has been referred to as the "junk food" hypothesis, which was proposed at an Alaska Sea Grant sponsored workshop in 1991. In short, this hypothesis addressed the possibility that food limitation could account for observed population declines in Alaskan marine mammals and birds (Alaska Sea Grant 1993). Recent counts of harbor seals are only 15% of counts made in the mid 1970's (Small et al. 2000). However, recent trend counts suggest that there have

been slight increases in population numbers in certain regions within Alaska. For example, population levels on Tugidak Island appear to be slightly increasing, or stabilizing, with estimated numbers exceeding 1200 during peak pupping counts during the late 1990's (Jemison and Kelly 2001). These numbers are up from a maximum count of approximately 650 seals in 1994 (Jemison and Kelly 2001). The population within Prince William Sound (PWS) appears to continue to decline at a yearly rate of 3.3% (Frost et al. 1999). While there is biochemical evidence that harbor seals in PWS are exposed to some physical, physiological, or environmental stress (Zenteno-Savin et al. 1997), no link has been made to nutrition.

Because of ongoing marine mammal and bird declines in Alaska, there is a need to establish a causal relationship between a series of population-wide health indicators and theoretical health problems resulting from food limitation. Hanks (1981) concluded that in a large vertebrate population measurements of the condition of an individual can be obtained and extrapolated to the population by estimating deposited fat reserves, adrenocortical hypertrophy, blood chemistry and hematology, urine hydroxypoline, and a body mass index. It should be noted that most of the indices used in this type of assessment have been collected from terrestrial mammals and may not apply to marine mammals. For example, Riney (1955) described the sequence of fat catabolism in terrestrial mammals experiencing a declining nutritional plane. Harder and Kirkpatrick (1994) proposed that to assess a nutritional index for a mammal it is necessary to estimate whole body fat by means of kidney fat index, marrow fat, and blood and urine characteristics. For free-ranging marine

mammals such as the harbor seal, blood chemistry and hematology, whole body fat, and morphometrics are the primary indices used to assess health (Fadely 1997).

When using blood chemistry as a measure of health it is important to identify boundary conditions or sources of variation in values within populations. Previous researchers have suggested that problems arise when trying to rank blood boundary conditions in order of importance, because each parameter is unique. Some of the earliest work in population assessment using blood chemistry data for gross nutritional status suggested that, without the quantification of a controlled study, the usefulness of blood studies is limited to describing reference ranges (Franzmann and Schwartz 1988). Because many blood characteristics have daily rhythms and change seasonally (Rosen and Renouf 1995, de Swart et al. 1995, Ryg et al. 1990, DelGuidice et al. 1992, Knick et al. 1993, Ferrer and Dobado-Barrios 1998) and with gender (Heidel et al. 1996), finding a correlation between health and a suite of blood chemistry parameters is difficult.

Changes in blood chemistry may also occur due to differences in diet, molt, lactation, or environmental perturbations. However, several researchers have determined that certain blood parameters have correlated well with nutritional status, and thus may be used as an index for health. Knick et al. (1993) analyzed blood from bobcats to assess nutritional status from animals occupying high, medium, or low prey density areas. While they found that there was a significant decline in serum insulin and cholesterol with low prey densities, red blood cell (RBC) counts, hemoglobin (Hb), and hematocrit (Hct) were determined to represent the best index

of change in nutritional status. Also, they reported a very large increase in blood urea nitrogen (BUN) and triglycerides in emaciated animals. In a study involving a sample of 300 bears, Franzmann and Schwartz (1988) found that Hb and Hct were the best blood parameters to assess condition. In a study involving harbor seals, de Swart et al. (1995) fed 22 animals PCB-contaminated herring for 125 weeks and found significant differences in Hct. While no significant differences were found in other hematological parameters, they did suggest that Hb, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin content (MCHC), and mean cell volume (MCV) all declined with age. This suggests that these hematology values may be influenced by age or development. Thompson et al. (1997) determined that harbor seals feeding in periods of "bad" prey years had significant differences in RBC counts and MCHC, but also mentioned that these changes in blood parameters may co-vary with age. Renouf and Noseworthy (1991) suggest that there is a relationship between total blubber and thyroid hormone levels in harbor seals. It appears from these studies that specific blood parameters are affected by various biotic and abiotic factors. However, it is not clear that there is one universally applicable index to assess health.

The second chapter of this thesis looks at the issue of blood as an index of population health by examining blood chemistry and morphology reference range values between two geographically close harbor seal pup populations in Alaska. Also, I consider the hypothesis that significant nutritional disorders could be affecting harbor seal pups, based on what is known regarding metabolite and

hematology changes in responses to potential nutritional deficiency in pinnipeds (Thompson et al. 1997, Rea et al. 1998).

Clinical chemistry and hematological values have been used to assess the nutritional and biomedical status of free ranging pinniped populations (Ronald et al. 1969, Geraci and Smith 1975, Castellini et al. 1993, Thompson et al. 1997). However, these studies did not usually account for the co-dependent nature of various blood parameters, and thus assessed health based on individual blood parameters or by comparing against previously reported values (Banish and Gilmartin 1988). While the importance of obtaining and understanding the physiological condition of a population is clear, health assessment in a population would be enhanced by understanding the health status of the individuals occupying the outer portions of the bell curve since values associated with this statistical region (outliers) are often suggestive of poor health (Rebar and Boon 1983, Vaughn 1999). While there is no single, generally accepted formal definition of an outlier, it is typically defined as an observation, which appears to be inconsistent with the remainder of the data set. Most outlier/discordancy tests are developed for differing circumstances, depending on data distribution, whether distribution parameters are known, and the number and type (upper or lower) of expected outliers. These tests, which primarily use 95% confidence intervals as criteria to determine which points are atypical, are primarily used on univariate samples where outliers may "stick out at the end" of the distribution. For instance, Fadely (1997) used blood chemistry parameters in a binomial model to determine the extent of individual outliers to

provide possible clues to population condition in harbor seals in Alaska. While promising, the use of this model would create an additive effect for "outliers", and could lead to erroneous results since blood variables are not independent. Instead, a model needs to be applied that distinguishes individual harbor seals from a suite of variables.

Chapter 3 focuses on developing a technique in which population "outliers" are graphically represented from plasma chemistry data and assessed for possible clinical implications. Using the same populations of harbor seal pups in Alaska described in Chapter 2, I establish a method to graphically represent outliers within a population to assess whether nutrition can be implicated in the decline in seal numbers in Alaska.

As in terrestrial systems, the use of captive marine mammals can provide valuable insight into the relative contribution of external influences on specific physiological responses. While controlled captive studies involving seasonal blood chemistry changes can represent baseline parameters for capture stress effects, they do not consider the influence of natural diets, energy expenditure, or the impact of natural perturbations (i.e. ENSO events)(de Swart et al. 1995). However, without incorporating captive studies, an in-depth examination of seasonal metabolic physiology essential to understanding the free-ranging population would not be feasible. There are many reports regarding seasonal and/or possible dietary influences on blood values in free-ranging pinnipeds (Trumble and Castellini 2002, Rea et al. 1998, Fadely 1997, Castellini et al. 1993, Costa and Ortiz 1982,

Schumacher et al. 1992). However, fewer data exist regarding changes in blood values in captive pinnipeds (De Swart et al. 1995, Nielson 1995, Ronald et al. 1969). In order to understand the spatial and temporal physiological responses in free-ranging marine mammals with regard to the environment, natural variation of environmental variables must be derived from captive models.

The primary objective of Chapter 4 is to identify the physiological variations expressed as differences in blood biochemistry and hematology levels as they are influenced by seasonal fluctuations and food quality. To do this I studied a group of captive harbor seals over 2 years. I also determined if changes in diet and/or season influenced morphology.

There have been several studies involving free-ranging marine mammals that have used changes in morphology as an index of body condition (Fadely 1997, Castellini et al. 1993, Calkins et al. 1998). Captive seal studies have also demonstrated that morphology (mass) is influenced by diet or season (Rosen and Trites 2000b, Ashwell-Erickson and Elsner 1981, Ronald et al. 1969). However, most studies reporting changes in morphology in captive marine mammals are short-term or a secondary focus of the research. To our knowledge, this represents the first study to focus on the possible influences of season and diet on blood chemistry, hematology and morphology in captive harbor seals.

The flexibility of the digestive system to accommodate and process nutrients may determine food selection and the nutritional niche occupied by an animal (Perrin 1994). Large aquatic carnivores such as pinnipeds, which feed while diving, must

contend with patchily distributed food sources, and long periods of searching interspersed with short periods of food consumption. Pinnipeds are generally considered opportunists while feeding (Brown and Mate 1983) and these piscivorous predators can experience multi-fold differences in energy intake rates based solely on the type and size of fish consumed (Anthony et al. 2000). Thus, it is possible for harbor seals to consume and process large amounts of lipid and protein in each feeding bout. Large meals require large capacity in the foregut with a concomitant ability to produce digestive secretions and absorb the products of degradation (Stevens and Hume 1995). Although endogenous enzymes can degrade the substrates from vertebrate prey, net uptake from the digestive tract may be limited by demand for secretions and by the time for digestion and absorption (Penry and Jumars 1987). As a result, digestive efficiency may be reduced by high rates of digesta flow due, to high rates of intake or low capacity of the digestive tract (Sibly and Calow 1986). Reports of digestive or assimilation efficiencies vary little among species of fish consumed by captive pinnipeds. Energy or lipid content of prey is, however, inversely related to digestibility in these animals (Ashwell-Erickson and Elsner 1981, Keiver et al. 1984, Fadely et al. 1990, Fisher et al. 1992, Rosen and Trites 2000a). This trade off between digestion and intake is of particular interest in declining populations because diet type could limit the animals' maintenance abilities (Rosen and Trites 2000b). Consequently, low fat prey, which has been described as "junk food", has been implicated in causing physiological and morphological stress in free-ranging animals (Calkins et al. 1998; Rosen and Trites 2000b; Trites and Jonker

2000). Short-term studies of Steller sea lions suggested that the sea lions were unable to maintain mass on a diet consisting exclusively of walleye pollock, but could when consuming a more lipid dense fish such as Pacific herring (Rosen and Trites 2000a).

Chapter 5 of this dissertation (Trumble, Barboza and Castellini 2003) examines changes in digestive efficiency that accompanied changes in nutrient loads for harbor seals. I hypothesized that increased feeding frequency would increase nutrient loads on seals and thus reduce retention time and the digestive efficiency of natural prey. Seals were fed two prey items (herring and pollock) of different lipid contents in two frequencies to simulate discontinuous feeding (once daily, low feeding frequency, low FF) or frequent feeding (four times each day, high FF) during winter, summer and autumn. Digestive performance was measured as voluntary food intake along with digesta flow of particulate and solute markers, and apparent efficiency of assimilating dry matter, lipid and nitrogenous compounds.

LITERATURE CITED

Alaska Sea Grant. 1993. Is it Food?: Addressing marine mammal and sea birds declines. Workshop Summary. Alaska Sea Grant Report 93–01.

Anthony J.A., D.D. Roby, and K. R. Turco. 2000. Lipid content and energy density of forage fishes from the northern Gulf of Alaska. Journal of Experimental Marine Biology 248:53-78

Ashwell-Erickson S, Elsner R (1981) The energy cost of free existence for Bering Sea harbor and spotted seals. In: Hood, D.W. and J.A. Calder (eds.), The Bering Sea Shelf: Oceanography and Resources, Vol. 2., pp.869-899. University of Washington Press.

Banish L.D., and W.G. Gilmartin. 1988. Hematology and serum chemistry of the young Hawaiian monk seal (*Monachus schauinslandi*). Journal of Wildlife Diseases 24:225-230.

Brown R.F., and B.R. Mate. 1983. Abundance, movements, and feeding habits of harbor seals, *Phoca vitulina*, at Netarts and Tillamook Bays, Oregon. Fisheries Bulletin 81:291-301.

Burns, J. M., S. J. Trumble, M. A. Castellini, and J. W. Testa. 1998. The diet of Weddell seals in McMurdo Sound, Antarctica as determined from scat collections and stable isotope analysis. Polar Biology 19:272-282.

Calkins D.G., E.F. Becker and K.W. Pitcher. 1998. Reduced body size of female Steller sea lions from a declining population in the Gulf of Alaska. Marine Mammal Science 14:232-244.

Castellini M.A., R.W. Davis, T.R. Loughlin and T.M. Williams. 1993. Blood chemistries and body condition of Steller sea lion pups at Marmot Island, Alaska. Marine Mammal Science 9(2):202-208.

Costa D.P., and C.L. Ortiz. 1982. Blood chemistry homeostasis during prolonged fasting in the northern elephant seal. American Journal of Physiology 242:R591-R595.

DelGiudice G.D., L.D. Mech, K.E. Kunkel, E.M. Gese, and U.S. Seal. 1992. Seasonal patterns of weight, hematology, and serum characteristics of free-ranging female white-tailed deer in Minnesota. Canadian Journal of Zoology 70:974–983.

de Swart R.L., P.S. Ross, L.J. Vedder, F.B.T.J. Bionk, P.J.H Reijnders, P.G.H Mulder and A.D. M.E. Osterhaus. 1995. Haematology and clinical chemistry values for harbour seals (*Phoca vitulina*) fed environmentally contaminated herring remain within normal ranges. Canadian Journal of Zoology 73:2035-2043.

Fadely B.S., G.A.J. Worthy, and D.P. Costa. 1990. Assimilation efficiency of northern fur seals determined using dietary manganese. Journal of Wildlife Management 54:246-251.

_____, 1997. Investigations of harbor seal *(Phoca vitulina)* health status and body condition in the Gulf of Alaska. Ph.D. Dissertation University of Alaska Fairbanks. 183 pp.

Ferrer, M., and P. Dobados-Berrios. 1998. Factors affecting plasma chemistry values of the Spanish Imperial Eagle, *Aquila adalberti*. Comparative Biochemistry and Physiology Part A 120:209-217.

Fisher, K.I., R.E.A. Stewart, R.A. Kastelein, and L.D. Campbell. 1992. Apparent digestive efficiency in walruses (*Odobenus rosmarus*) fed herring (*Clupea harengus*) and clams (*Spisula* sp.). Canadian Journal of Zoology 70:30-36.

Franzmann A.W. 1985. Assessment of nutritional status. In: Bioenergetics of wild herbivores. R. J. Hudson and R. G. White eds. CRC press. pp. 239-259.

_____, and C.C. Schwartz. 1988. Evaluating condition of Alaskan black bears with blood profiles. Journal of Wildlife Management 52:63-70.

Geraci, J.R., and T.G. Smith. 1975. Functional hematology of ringed seals (*Phoca hispida*) in the Canadian Arctic. Journal of Fisheries Research Board of Canada 32:2559-2564.

Hanks, J. 1981. Characterization of population condition. In: Dynamics of large mammal populations. Fowler, C.W., and T.D. Smith, eds. pp. 47-73.

Harder, J.D. and R.L. Kirkpartrick. 1994. Physiological methods in wildlife research. In: Research and management techniques for wildlife habitats. Bookhout, T.A. ed., pp. 275-306.

Harvey, J.T. 1989. Assessment of errors associated with harbor seals, *Phoca vitulina*, faecal samples. Journal of Zoology London. 219:101-111.

Heidel J.R., L.M. Philo, T.F. Albert, C.B. Andreasen, and B.V. Stang. 1996. Serum chemistry of bowhead whales (*Balaena mysticetus*). Journal of Wildlife Diseases 32:75-79.

Jeffries, S.J. 1986. Seasonal movements and population trends of harbor seals, *Phoca vitulina richardsi* in the Columbia River and adjacent waters of Washington and Oregon: 1976-1982. Report to the U.S. Marine Mammal Comm., Contract No. MM2079357-5.

Jemison, L.A., and B.P. Kelly. 2001. Pupping phenology and demography of harbor seals (*Phoca vitulina richardsi*) on Tugidak Island, Alaska. Marine Mammal Science 17:585-600.

Keiver, K.M., K. Ronald, and F.W.H. Beamish. 1984. Metabolizable energy requirements for maintenance and faecal and urinary losses of juvenile harp seals (*Phoca groenlandica*). Canadian Journal of Zoology 62:1751-1756.

Knick, S.T., E.C. Hellgren, and U.S. Seal. 1993. Hematologic, biochemical, and endocrine characteristics of bobcats during a prey decline in southeastern Idaho. Canadian Journal of Zoology 71:1448-1453.

Nielsen, J. 1995. Immunological and hematological parameters in captive harbor seals (*Phoca vitulina*). Marine Mammal Science 11:314-323.

Ohtani, K., and T. Azumaya. 1995. Influence of interannual changes in ocean conditions on the abundance of walleye pollock (*Theragra chalcogramma*) in the eastern Bering Sea. In: Beamish, R.J., (ed). Climate Change and northern fish populations. Canadian Special Publication in Fisheries and Aquatic Sciences 121:87-95.

Penry, D.L., and P.A. Jumars. 1987. Modeling animal guts as chemical reactors. American Naturalist 129:69-96.

Perrin, M.R. 1994. Herbivory and niche partitioning. In: The digestive system in mammals: food, form, and function. Chivers, D.J., and Langer, P. eds. Cambridge University Press, Cambridge, United Kingdom. pp. 128-149

Pitcher, K.W. 1977. Population productivity and food habits of harbor seals in the Prince William Sound Copper River Delta area Alaska. Report to U.S. Marine Mammal Comm. Report No. MMC-75/03. 36 pp.

_____,1980. Food habits of the harbor seal (*Phoca vitulina richardsi*) in the Gulf of Alaska. Fisheries Bulletin 78:544-549.

_____, 1990. Major decline in the number of harbor seals (*Phoca vitulina richardsi*) on Tugidak Island, Gulf of Alaska. Marine Mammal Science 6:121-134.

Rea, L.D., M.A. Castellini, B.S. Fadely and T.R. Loughlin. 1998. Health status of young Alaska Steller sea lion pups as indicated by blood chemistry and hematology. Journal of Comparative Biochemistry and Physiology A 120:617-623.

Rebar, A.H. and G.D. Boon. 1983. A case-oriented approach to small animal biochemical profiling. Ralston Purina Co. St. Louis, MO. 104 pp.

Renouf, D. and E. Noseworthy. 1991. Changes in food intake, mass and fat accumulation in association with variations in thyroid hormone levels of harbour seals (*Phoca vitulina*). Canadian Journal of Zoology 69:470-479.

Riedman, M. 1990. The Pinnipeds: Seals, Sea Lions, and Walruses. University of California Press, Berkeley. 439 pp.

Riney, T. 1955. Evaluating condition of free-ranging red deer (*Cervus elaphus*) with special reference to New Zealand. New Zealand Journal of Science 36:429-463.

Ronald, K., M.E. Foster, and E. Johnson. 1969. The harp seal, *Pagophilus groenlandicus* (Erxleben, 1777). II. Physical blood properties. Canadian Journal of Zoology 47:461-468.

Rosen, D.A.S., and A.W. Trites. 2000a. Digestive efficiency and dry-matter digestibility in Steller sea lions fed herring, pollock, squid, and salmon. Canadian Journal of Zoology 78:234-239.

_____, and A.W. Trites. 2000b. Pollock and the decline of Steller sea lions: testing the junk-food hypothesis. Canadian Journal of Zoology 87:1243-1250.

_____, and D. Renouf. 1995. Variation in the metabolic rates of harbour seals. In; Whales, seals, fish and man. Blix, A.S. ed. pp. 12-24.

Ryg, M., T.G. Smith, and N.A. Oritsland. 1990. Seasonal changes in body mass and body composition of ringed seals (*Phoca hispida*) on Svalbard. Canadian Journal of Zoology 68:470-475.

Seal, U.S., Verme, L.J., and Ozoga, J.J. 1981. Physiologic values. Tall Timbers Research Station Miscellaneous Publication No. 7. 36 pp.

Schumacher, U., G. Rauh, J. Plotz, and U. Welsch. 1992. Basic biochemical data on blood from Antarctic Weddell seals (*Leptonychotes weddelli*): ions, lipids, enzymes, serum proteins and thyroid hormones. Journal of Comparative Biochemistry and Physiology A 102:449-451.

Sibly, R.M., and P. Calow. 1986. Physiological ecology of animals: an evolutionary approach. Blackwell, Oxford, UK.

Slater, S.E., and H. Markowitz. 1983. Spring population trends in *Phoca vitulina richardsi* in two Central California coastal areas. California Fish and Game Bulletin 69:217-226.

Small, R.J. 2000. Executive Summary In: Harbor Seal Investigations, Alaska Department of Fish and Game Annual Report, NOAA # NA87FX0300. Small, R.J. (P.I). pp. 324-344.

Stevens, C.E., and I.D. Hume. 1995. Comparative physiology of the vertebrate digestive system. Cambridge Univ. Press. 2nd ed. 398 pp.

Thompson, P.M., D.J Tollit, H.M. Corpe, R.J. Reid, and H.M. Ross. 1997. Changes in haematological parameters in relation to prey switching in a wild population of harbour seals. Functional Ecology 11:743-750.

Trites, A.W., and R.A.H. Jonker. 2000. Morphometric measurements and body composition of healthy and starving Steller sea lion pups (*Eumetopias jubatus*). Aquatic Mammalogy 26(2):151-157.

Trumble, S.J., P.S. Barboza and M.A. Castellini. 2003. (in press). Digestive constraints on an aquatic carnivore: effects of feeding frequency and prey composition on harbor seals. Journal of Comparative Biochemistry and Physiology B.

_____, and M.A. Castellini. 2002. Blood chemistry, hematology, and morphology of wild harbor seal pups in Alaska. Journal of Wildlife Management 66(4):1197-1207.

Vaughn, G. 1999. Understanding and evaluating common laboratory tests. Appleton and Lange. Stamford CT. 678 p.

Zenteno-Savin, T., M.A. Castellini, L.D. Rea, and B.S. Fadely. 1997. Plasma haptoglobin levels in threatened Alaskan pinniped populations. Journal of Wildlife Diseases 33:64-71.

2

BLOOD CHEMISTRY, HEMATOLOGY AND MORPHOLOGY VALUES OF WILD HARBOR SEAL PUPS FROM DECLINING AND STABLE POPULATIONS IN ALASKA[1]

ABSTRACT

This study was designed to compare blood chemistry and morphology reference range values between two harbor seal pup populations in Alaska, one population in continued decline, Prince William Sound, and another population in recent increase, Tugidak Island. There were significant site-specific differences in five of the eight mean hematology values as well as eleven plasma chemistry values. We also determined significant year-to-year variability in eight (36%) mean plasma chemistry values. These results form the largest available field-based blood reference database for harbor seal pups. They demonstrate that blood values can vary on the population scale and that health assessment or eco-physiological studies involving blood chemistry must utilize an appropriate set of reference values for valid comparisons.

[1] Trumble S.J. and M.A. Castellini. 2002. Blood chemistry, hematology and morphology values of wild harbor seal pups from declining and stable populations in Alaska. Journal of Wildlife Management 66(4):1197-1207.

INTRODUCTION

Comparative blood chemistry and morphology is a valuable clinical tool for evaluating health and has been used to assess physiological and pathological condition in free-ranging and captive marine mammals (Castellini et al. 1993, Thompson et al. 1997, Calkins et al. 1998). Recent declines in harbor seal populations in Alaska have emphasized the need to obtain health index biomarkers for comparative purposes. By definition, reference values provide a range for each particular blood parameter within which the majority (i.e. 95%) of healthy animals lie. Values outside of this range often suggest compromised health. While often compared, blood parameters can vary according to nutritional state and/or circadian rhythm, population sample size, laboratory method used, gender, disease state, and developmental stage of the animal (Sunderman 1975, Solberg 1984, Garcia-Rodriguez et al. 1987,Thompson et al. 1992, Fadely 1997, Burns et al. 1998). Therefore, when assessing health, it is essential that rigorous blood chemistry reference ranges be established for the specific groups or populations of animals.

The harbor seal (*Phoca vitulina*) population in Alaska has declined by nearly 90% in some regions over the past three decades (Frost *et al.* 1999). While there are modest signs of recovery in certain regions, the population within Prince William Sound (PWS) is still declining at a yearly rate of 3.3% (Frost *et al.* 1999). Harbor seal counts on Tugidak Island, just south of Kodiak Island, once estimated at nearly 20,000 during the early 1950's and 1960's, reached a low maximum count in 1994 of

approximately 650 seals (Jemison and Kelly 2001). Recent population levels on Tugidak Island appear to be slightly increasing, or stabilizing, with estimated numbers exceeding 1200 during peak pupping counts on the southwest beach during the late 1990's (Jemison and Kelly 2001).

This study was designed to compare blood chemistry and morphology reference range values among two harbor seal pup populations in Alaska, one population in continued decline (PWS) and another in an area of recent increase (Tugidak Island). Also, we considered the recent hypothesis that significant nutritional disorders could be affecting harbor seal pups during the first weeks of life. This theory links the continued decline of PWS harbor seals to gross nutritional status based on what is known regarding metabolite and hematology changes in responses to potential nutritional deficiency in pinnipeds (Thompson et al. 1997, Rea et al. 1998).

While several studies have addressed blood chemistry, hematological and morphological reference values for harbor seal adults (McConnel and Vaughn 1983, de Swart et al. 1995, Fadely 1997, Kopec and Harvey 1995) few papers have reported results of pups (Dierauf and Dougherty 1983), especially from wild populations.

Study area

Seals pups were captured in two locations, Tugidak Island and within Prince William Sound, Alaska. Tugidak Island is located 40 miles southwest of Kodiak Island (56^0 30' N, 154^0 40' W). Approximately 150 miles separate these locations.

METHODS

Within Prince William Sound, harbor seals (adults and pups) were live-captured by net entanglement on haul-out sites using methods previously described by Frost et al. (1995). Pups on Tugidak Island were captured opportunistically on sandy beaches with large landing nets. All pups were captured between 25 June and 2 July (median pups age was approximately 2 weeks) during all years sampled in each location. Onset of pupping was known for each sample year on Tugidak Island (Jemison and Kelly 2001), however, these data were not available in PWS and thus we assumed similar onset of pupping. Seal pups that appeared healthy by gross visual observation (no signs of external injuries, lesions, or malnutrition (axial blubber thickness < 10mm)) were transported to ship or shore and manually restrained until all samples were collected. Precise age could not be determined for individual pups from either site, however, we were careful to avoid pups with an umbilicus and sample only pups with milk teeth.

Morphometrics

Mass was measured using an electronic load cell (+ 0.5 Kg, Ohaus I-20W); girths at three locations (maximum, axial, and hip) and standard length (SL; straight-line distance between tip of nose and tip of tail) were measured using a tape measure (± 1 cm) with the seal positioned dorsal side up (Castellini and Kooyman 1990, Castellini and Calkins 1993). Blubber thickness was measured at three locations (dorsally) at each girth measurement location using a portable ultrasonic unit (Gales

and Burton 1987, Scanoprobe II, Model 7310, Scanco, Inc.). Blubber thickness relative to body thickness was calculated by dividing the blubber thickness by the body radius at that girth site. Gender for each pup was determined by the presence or absence of a penile opening.

Blood collection and analysis

Blood samples were drawn from the extradural intervertebral vein using 18G X 3.5 inch (1.2 X 90mm) spinal needles into heparinized, EDTA, or serum evacuated glass blood collection tubes (Vacutainer®). Collected blood samples were chilled until their return to ship or lab (<5h). Complete blood counts of white and red blood cells and differential counts were determined from whole blood smears made on microscope slides in the field and were analyzed by technicians at Fairbanks Memorial Hospital (FMH) using 100 cells/high-powered light microscopy methods. Hematocrit (Hct) was determined in the field on freshly drawn blood by using a microcrit centrifuge (Compur M1100). Samples for hemoglobin (Hb) analysis were prepared by pipetting 10 uL whole blood into 2.5 mL Drabkin's reagent for subsequent photometric determination (Sigma® kit #525). The remaining whole blood was centrifuged and the plasma and serum were separated and stored at -196°C (liquid nitrogen dry shipper). Plasma samples were sent to FMH for assessment of standard panel of clinical blood chemistries including sodium (Na), potassium (K), chloride (Cl), calcium (Ca), phosphate (P), cholesterol (CHOL), glucose (GLU), protein (TP), blood urea nitrogen (BUN), albumin (ALB), creatinine (CR), globulin

(GLOB), bilirubin (BILI), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine phosphokinase (CK), gammaglobulin transferase (GGT), and alkaline phosphatase (AP). All assays were performed by FMH technicians using automated machine analysis (Kodak Ektachem 550 Analyzer). FMH has provided research blood chemistry analysis for veterinary and animal programs at the University of Alaska Fairbanks for over 10 years.

Statistical analysis

Field collections of serum or plasma were visually examined for gross hemolysis or lipemia. All blood parameters within hemolyzed or lipemic samples were statistically compared with blood parameters from non-compromised samples (ANOVA).

A Pearson correlation matrix was used to measure similarities among morphology parameters for pups from each population sampled. Blood chemistry, hematology, and morphology parameters were analyzed for statistical differences between region and gender for all harbor seal pups using ANOVA techniques. Transformations were used to improve normality when appropriate, however non-parametric statistics were used on non-normal data. To address possible differences in nutrition quality (Thompson et al. 1997) we evaluated hematology variables with relative blubber thickness for differences among season and between location and gender (MANOVA with Hb, Mean Corpuscular Hemoglobin Content (MCHC) and relative axial blubber thickness as dependant variables and gender, location and year

as independent variables). Relationships between hematocrit and hemoglobin values along with standard length to mass measurements were also assessed using least linear regression techniques. Regression coefficients were compared and tested for significance using ANOVA. A Kolmolgorov-Smirnoff Probability Test was used to assess normality for all statistical tests. A Bonferroni comparison test was performed to identify significant differences in blood chemistry, hematology, of morphology measurements among pups between regions, genders and years. Reference ranges for blood chemistry and hematology parameters for harbor seal pups were calculated as the interval between the 2.5 and 97.5 percentiles (mean $\pm$ 2 standard deviations). Alpha levels (α) were placed at 0.05 for all statistical tests. All statistical tests were performed using SYSTAT® (ver. 10) and SPSS software (ver. 9).

RESULTS

A total of 174 pups were captured from Tugidak Island (n = 95) and PWS (n = 79) between 25 June and 2 July 1997 and 2000. Significantly more males were captured on Tugidak Island (males = 51, females = 44) than from within PWS (males = 37, females = 42; P = 0.007).

Morphology

PWS pups were significantly heavier than pups captured from Tugidak Is. at time of capture for each year (Fig. 2.1a, Table 2.1). Mean mass of male pups also was significantly greater than females at time of capture (pooled data, df = 172, p = 0.02; males, n = 95, x = 29.1 kg, SD = 4.7; females, n = 79, x = 26.9 kg, SD = 4.2, Table 2.1). While there was no site-specific difference in the standard length of

harbor seal pups, mean SL for males was greater than females (pooled data, df = 172, p = 0.001; males, n = 95, x = 94.8 cm, SD = 5.9; females, n = 79, x = 92.1 cm, SD = 4.7, Table 2.1.). PWS pups had greater mean axial and maximum girth measurements when compared to pups captured on Tugidak Island (Table 1). There were also gender-related differences in mean axial girth measurements (pooled data, df = 172, p = 0.03; males, n = 95, x = 83.6 cm, SD = 6.2; females, n = 79, x = 81.0, SD = 9.0).

Correlations of morphology values for pooled harbor seal pups from PWS and Tugidak Island are presented in Table 2.2. For each population of harbor seal pups the best predictor of body mass was maximum girth (Pearson correlation coefficient for pooled data, r = 0.836, Table 2). A linear regression between maximum girth and body mass reveals:

$$\textit{Mass (kg)} = 0.672\ \textit{(max girth)} - 28.16$$

for pups sampled in Alaskan waters (N = 174, $r^2 = 0.69$, Fig 2). The maximum girth to mass relationship in pups is comparable to previously established condition indices used in pinniped adults (mass to SL*(Axial Girth)^2, $r^2 = 0.66$; Castellini et al. 1990, Castellini et al. 1993)

Absolute blubber thickness was significantly greater for pups captured within PWS when compared to seal pups from Tugidak Island (Table 2.1). Relative mean blubber thickness, which is blubber thickness divided by the body radius at a given girth, at maximum girth (p = 0.000, df = 71, Tugidak, n = 44, x = 0.52, SD = 0.08; PWS, n = 29, x = 0.61, SD = 0.11) and hip (p = 0.000, df = 51, Tugidak, n = 24, x =

0.60, SD = 0.21; PWS, n = 29, x = 0.77, SD = 0.08) were also greater for pups within PWS; however, there was no significant difference among relative axial blubber depths among populations (p = 0.484, df = 89, Tugidak, n = 62, x = 0.54, SD = 0.28; PWS, n = 69, x = 0.58, SD = 0.08).

Hematology

Table 2.3 contains the mean values and reference ranges for all hematology data collected from Tugidak Island and PWS harbor seals pups. The degree of hemolysis or lipemia had no effect on hematology values therefore all blood samples collected were used in subsequent analysis. Not all leukocyte parameters were obtained from blood samples (i.e. basophils), therefore statistical analysis was limited to eight of the ten parameters collected. There were significant site-specific differences in five of the eight mean hematology values (Table 2.3). No significant gender-related differences were detected among populations in mean hematology parameters therefore all statistical analysis was performed on pooled data within each location. Pups captured on Tugidak Is. had significantly lower mean Hct, Hb and MCHC (Hb/[Hct/100]) values than pups captured within PWS (Table 2.3, Fig. 2.3). A least linear relationship between Hct and Hb among populations revealed no significant difference in regression coefficients (p = 0.84, pooled r^2 = 0.68; Fig. 2.4a,b), however, gender-related differences in Hct/Hb regression coefficients were evident from pups on Tugidak Island (p = 0.04) and not in pups captured within PWS.

Monocyte values were significantly elevated in Tugidak Island pups while eosinophil levels were elevated in PWS pups (Table 2.3).

There was no statistical relationship between Hb and MCHC with relative axial blubber thickness among years, location, or gender (MANOVA; Gender, p = 0.53 df = 1; Year, p = 0.243, df = 3; Location, p = 0.174, df = 1).

There was no difference between locations in NRBC counts for harbor seal pups (Table 2.3), however PWS seals had increased values during 1997 (x = 10.2/100WBC's) when compared to 2000 (x = 8.7/100WBC's; p =0.043, df = 38)

Plasma chemistry

It was determined that cholesterol (Tugidak, P = 0.01; PWS, P = 0.02), ALT (PWS, P = 0.05) and creatinine (PWS, P = 0.04) values from lipemic samples were significantly different from non-compromised samples thus were not used in subsequent statistical analysis. Gross hemolysis affected ALT, albumin, AST, BUN, glucose, bilirubin, K, LDH, CPK and GGT values and thus hemolyzed samples were omitted from subsequent plasma chemistry comparisons.

Table 2.4 contains the mean values and reference ranges for all plasma chemistry data collected from Tugidak Island (n = 80) and PWS (n = 72) harbor seals pups. Eleven mean plasma chemistry values (54%) had significant site-specific differences with all but one (ALT) significantly higher in pups captured on Tugidak Island (Table 2.4). There were gender-specific differences in mean glucose and creatinine levels (GLU, males, x = 2.3 mg/dL SD = 0.06; females, x = 2.1 mg/dL,

SD = 0.05; P = 0.01; CREA, males, x = 0.74 mg/dL, SD = 0.12; females, x = 0.69 mg/dL, SD = 0.15; P = 0.02). There was significant year-to-year variability in eight (36%) mean plasma chemistries; Na, Cl, P, CREA, TP, GLOB, AST, GLU (Table 2.4, Fig. 2.5).

DISCUSSION

This study yielded blood chemistry, hematology and morphometric data from harbor seal pups from two geographically distinct areas in Alaska. While blood parameters are routinely used as comparative bio-markers in both marine and terrestrial mammal research programs, we propose each population's blood status, which may be normal in the statistical sense, may fall outside the range of normal in another population. Many blood chemistry values obtained from pups during this study were outside the reference range values of previously published adult harbor seals (Bossart and Dierauf 1990, Roletto 1993, de Swart et al. 1995, Fadley 1997, McConnel and Vaughn 1983, Thompson et al. 1997, Kopec and Harvey 1995). We also observed between-location differences in greater than half of all mean blood parameters measured, which translated into significant reference range differences between a stable and declining population. Because of the difficulty of linking these statistical differences and potential health perturbations to individual blood parameter differences (is one value high or the other value low?), we suggest establishing and using blood parameters as indices of temporal change (each population is its own control), especially the parameters influenced by external

factors (i.e. nutrition). Therefore, while this study has established reference ranges for a large database of pups in Alaska, the statistical differences (biologically significant?) identified between a stable and declining population should provide warning against comparative health assessment by comparing "normal" values from other populations of seals.

Body mass and relative body fat values were significantly lower in harbor seal pups captured on Tugidak Island. Trites and Bigg (1992) suggested that body length and mass along with condition of the Northern fur seals fluctuated due to the availability of prey while Calkins et al. (1998) proposed that Steller sea lion body size mirrored prey availability. However, because harbor seal pups rapidly gain mass over a short lactation period and potentially lose mass during initial weaning (Bonner 1984), body mass and relative fat may not be an acceptable comparative measure among populations.

There were site, gender and yearly differences in five hematological parameters in harbor seal pups captured during this study. It has been previously reported that Hb and MCHC in Atlantic harbor seals can decrease during periods of "bad" prey quality or quantity (Thompson et al. 1997, de Swart et al. 1995). During this study we witnessed yearly decreases in Hb and MCHC in both seal populations, although we found no relationship relating these hematological parameters with changes in body fat among years, gender or location. In other words, relative axial body fat remained constant despite a decrease in Hb and MCHC. Therefore, unlike the Thompson et al. (1997) findings with Atlantic harbor seals, we did not see a

relationship between body condition and hemoglobin levels. Hematology values may also be a function of age and handling of pups (de Swart et al. 1995, Thompson et al. 1997, Burns et al. 1998). Age-related changes in Hct in pinnipeds have been well documented in relation to diving physiology and handling conditions (Castellini et al. 1986, 1993, 1996). While we captured pups from each location during identical sampling periods (25 June- 2 July), initial pupping dates may have differed by as much as a week (Jemison and Kelly 2001), which could produce significant differences in morphological and certain hematological parameters (J. Burns pers. comm.). A difference in age may have resulted in the among year NRBC difference found in PWS pups. NRBC's are precursors to mature erythrocytes and the presence of these cells in the blood of very young human infants is normal (Vaughn 1999). Increased NRBC's may be indicative of anemia, however, we do not have corroborative data to confirm this. While we are beginning to understand the developmental and physiological changes that pinnipeds undergo during the first two weeks of life, it would be unreasonable to hypothesize what blood parameters change in response to development or environment since there are no studies to date of these changes in wild populations of harbor seals during this physiological transition period.

There were site-specific differences in plasma chemistry parameters including increased electrolyte levels (Na, K, Cl) in Tugidak Island harbor seal pups. Measurements of electrolytes have been used to provide information on the water balance of terrestrial mammals, with higher than reference range levels resulting

from dehydration (DelGiudice et al. 1987). Seals apparently overcome problems regarding food and water limitations during lactation by foraging (Boness et al. 1994) and while it also has been reported that electrolyte levels in a pup may reflect the nutritional state of the mother (Schweigert 1993), we have no data to support this. Previous data on adult harbor seals in Alaska suggest site and gender-specific differences in mean electrolyte levels (Fadely 1997). While our pup values are higher than reported adult values, it is unlikely that a fluid deficit of chronic physiological significance was manifested during the suckling period. In suckling elephant seal pups, plasma water values are slightly elevated compared to the weaned state and the animals are able to maintain water balance even when completely fasting from external food or water (Castellini et al. 1990).

We found significantly higher alkaline phosphatase values in Tugidak Island pups, which may imply developmental or age differences between the populations (Hall 1998, Vaughn 1999). Alkaline phosphatase is known to increase during periods of bone growth in terrestrial neonates and has been reported to decrease with age in phocid pups (Bossart and Dierauf 1990, Hall 1998). Schweigert (1993) reported that AP levels decreased approximately 40% from the period of suckling to weaning in grey seals.

Plasma BUN, creatinine and protein values are influenced by dietary quality and also hydration state in mammals (Schweigert 1993, Vaughn 1999). When compared to Tugidak Is. pups, PWS pups exhibited decreased plasma BUN, creatinine, protein and globulin values. While BUN is used as an indicator of fasting or starvation in

adult marine mammals (Rea et al. 1998), Worthy (1982) observed no significant differences in circulating levels of BUN, creatinine and protein during the postweaning fast when compared with the onset of feeding in harp seal pups. However, Rea et al. (1998) noticed elevated levels of BUN in Gulf of Alaska Steller sea lion pups (*Eumatopias jubatus*) and suggested possible extended foraging trips by the female as a potential cause. The elevation in mean plasma creatinine values observed in the comparatively leaner and lighter Tugidak Island pups is not in agreement with previous research that suggests creatinine production and excretion are directly related to muscle mass (Vaughn 1999). However, given that blubber thickness was greater in PWS pups, it may be possible that pups from Tugidak Island had increased relative muscle mass although in the absence of body composition data (i.e. D_2O) this cannot be confirmed. During this study, our intention was to collect blood and morphology samples from two distinct harbor seal populations (based on satellite telemetry data, R. Small pers comm) in Alaska during the same sampling period. While we accomplished this goal (median date of capture no greater than two days difference in any given year, and four days among all years sampled), we assumed similar age makeup of pregnant females and thus captured pups between populations. It has been reported that changes in the age makeup in pinniped populations can influence the timing of pupping, as parturition tends to be earlier in older female seals (Jemison and Kelly 2001). While there are recent data suggesting that maximum pup counts on Tugidak Island have not changed since 1994, little is known regarding the pupping phenology of PWS harbor seals. Therefore, we must

acknowledge that differences in these blood and morphology variables may have arisen as a result of a difference in pupping dates between populations. Also, changes in morphological and hematological parameters such as Hct, Hb and MCHC (J. Burns pers comm), as well as blood chemistry parameters such as AP, bilirubin, and phosphorus (Schweigert 1993, Hall 1998) may occur within days in harbor seal pups during their intense lactation period. However, while blood and hematology parameters can change with age, gender and nutrition, we cannot discount the possibility that these differences are linked to a metabolic profile or identity of the separate seal populations.

Development of reference ranges for free-ranging Gulf of Alaska harbor seal pups permits examination of clinical blood panels with more confidence than would have been possible utilizing ranges published from adult values, small sample sizes, or from captive or free-ranging seals from outlying geographic regions. Also, analysis of blood and morphological parameters may provide insight on developmental or age differences between populations. It is possible that diet and/or developmental status quality played a role in the differences in blood values observed among the populations. However, we must warn against interpreting health status between populations by statistically comparing singular or even multiple blood parameters. A better approach in studying health changes may be to analyze within population temporal differences. While this study was not designed to examine yearly differences in blood parameters, researchers should be aware of the site,

gender-specific and yearly variability associated with many blood parameters in harbor seal pups.

ACKNOWLEDGMENTS

This publication is the result of research sponsored by the Alaska Sea Grant College Program with funds from the National Oceanic and Atmospheric Administration Office of Sea Grant, Department of Commerce, under grant no. NA 86RG0050 (project no. R/08-08), and from the University of Alaska with funds appropriated by the state. We would like to thank Dr. R. Small for his contributions and assistance throughout the collection phase of this project (Alaska Department of Fish and Game permits #1000 and #358-1585 (NOAA Grant NA87FX0300)). This research was conducted with authorization from the University of Alaska Institutional Animal Care and Use Committee. We also would like to thank K. Frost and her crew of the Alaska Department of Fish and Game for providing blood samples from harbor seal pups from locations within Prince William Sound Alaska between 1997-2000. We also thank Dr. J. Burns for her assistance and helpful comments. We would like to acknowledge The Elmer Rasmuson Fisheries Fellowship for support throughout this project. We would like to thank the following for their assistance in this project, K. Wynne, J.M. Castellini, T. Mau, L. Jemison, J. Jemison, K. Hastings, S. Crowley and R. Daniel. This manuscript was greatly improved by the helpful comments from two anonymous reviewers.

LITERATURE CITED

Bonner, W. N. 1984. Lactation strategies in pinnipeds: Problems for a marine mammalian group. Symposium of Zoological Society of London 51:253-272.

Boness D. J, Bowen W.D. and O.T. Oftedal. 1994. Evidence of a maternal foraging cycle resembling that of otariid seals in a small phocid, the harbor seal. Behavioral Ecology and Sociobiology 34:95-104.

Bossart, G. D., and L. A. Dierauf. 1990. Marine mammal clinical laboratory medicine. In: L.A. Dierauf (eds). CRC Handbook of Marine Mammal Medicine: Health, Disease and Rehabilitation. CRC Press, Boca Raton, Florida. Pp. 1-52.

Burns, J. M., Trumble, S. J., Castellini, M. A. and J. W. Testa. 1998. The diet of Weddell seals in McMurdo Sound, Antarctica as determined from scat collections and stable isotope analysis. Polar Biology 19: 272-282.

Calkins, D.G., Becker, E. F., and K. W. Pitcher. 1998. Reduced body size of female Steller sea lions from a declining population in the Gulf of Alaska. Marine Mammal Science 14(2):232-244.

Castellini, J. M., Castellini, M. A., and M. B. Kretzmann. 1990. Circulatory warter concentration in suckling and fasting northern elephant seal pups. Journal of Comparative Physiology B 160:537-542.

Castellini, M. A., Costa, D. P., and A. Huntley. 1986. Hematocrit variation during sleep apnea in elephant seal pups. American Journal of Physiology 251: R429-R431.

_____, and G.L. Kooyman. 1990 Length, girth, and mass relationships in Weddell seals (*Leptonychotes weddellii*). Marine Mammal Science 6(1):75-77.

_____, Davis R. W., Loughlin T. R. and T. M. Williams. 1993. Blood chemistries and

body condition of Steller sea lion pups at Marmot Island, Alaska. Marine Mammal Science 9(2):202-208.

_____, and D. Calkins. 1993. Mass estimates using body morphology in Steller sea lions. Marine Mammal Science 9:48-54.

Castellini, J. M., Meiselman, H. J. and M. A. Castellini. 1996. Understanding and interpreting hematocrit measurements in pinnipeds. Marine Mammal Science 12:251-264.

DelGiudice, G. D., Mech, L. D., Seal, U. S., and P. D. Karns. 1987. Effects of winter fasting and refeeding on white-tailed deer blood profiles. Journal of Wildlife Management 51:134-145.

de Swart, R. L., Ross P. S., Vedder L. J., Bionk F. B. T. J., Reijnders P. J. H, Mulder P. G. H. and A. D. M. E. Osterhaus. 1995. Haematology and clinical chemistry values for harbour seals (*Phoca vitulina*) fed environmentally contaminated herring remain within normal ranges. Canadian Journal of Zoology 73: 2035-2043.

Dierauf, L. A., and S. A. Dougherty. 1983. Early evaluation of neonatal harbor seal (*Phoca vitulina richardsi*) health status 1: Preliminary report. J. Zoo An. Med. 14:138-144.

Fadely, B. S. 1997. Investigations of harbor seal (*Phoca vitulina*) health status and body condition in the Gulf of Alaska. Ph.D. Dissertation Univ. Alaska Fairbanks. 183 pp.

Frost, K.J., L.F. Lowry and J. Ver Hoef. 1995. Habitat use, behavior and monitoring of harbor seals in Prince William Sound, Alaska. Annual Rep. For *Exxon Valdez* Oil Spill Restoration Project (Restoration Projects 94064 and 94320-F), Alaska Dept. of Fish and Game, Wildlife Conservation Division, Fairbanks. 87pp.

_____, L.F. Lowry and J. Ver Hoef. 1999. Monitoring trends of harbor seals in Prince William Sound, Alaska, after the *Exxon Oil* Spill. Marine Mammal Science 15:494-506.

Gales N. J. and H. R Burton. 1987. Ultrasonic measurement of blubber thickness of the southern elephant seal, *Mirounga leonina* (Linn.). Australian Journal of Zoology 35:207-217.

Garcia-Rodriguez, T. Ferrer M., Carillo J. C. and J. Castroviejo. 1987. Circadian rhythms of determined blood chemistry values in Buzzards and Eagle Owls. Comparative Biochemistry and Physiology 88A: 663-669.

Hall, A. J. 1998. Blood chemistry and hematology of gray seal (*Halichoerus grypus*) pups from birth to postweaning. Journal of Zoology and Wildlife Medicine 29(4):401-407.

Jemison, L. A., and B. P. Kelly. 2001. Pupping phenology and demography of harbor seals (*Phoca vitulina richardsi*) on Tugidak Island, Alaska. Marine Mammal Science 17(3):585-600.

Kopec, A. D., and J. T. Harvey. 1995. Toxic pollutants, health indices, and population dynamics of harbor seals in San Francisco Bay, 1989-1992. Final Rep., Moss Landing Marine Laboratories, Moss Landing, CA. 138pp.

McConnel, L. C., and R. W. Vaughn. 1983. Some blood values in captive and free-living common seals (Phoca vitulina). Aquatic Mammalogy 10:9-13.

Rea, L.D., Castellini M. A., Fadely B. S. and T.R. Loughlin. 1998. Health status of young Alaska Steller sea lion pups as indicated by blood chemistry and hematology. Comparative Biochemistry and Physiology A 120: 617-623.

Rolleto, J. 1993. Hematology and serum chemistry values for clinically healthy and sick pinnipeds. Journal of Zoology and Wildlife Medicine 24:145-157.

Schweigert, F. J. 1993. Effects of fasting and lactation on blood chemistry and urine composition in the grey seal (*Halichorus grypus*). Comparative Biochemistry and Physiology 105A(2): 353-357.

Solberg, H. E. 1984. The theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits. Clin. Chim. Acta. 137:95F-113F.

Sundermann, F.W. Jr. 1975. Current concept of "normal values", "reference values" and "discrimination values" in clinical chemistry. Clinical Chemistry 21:1873-1877.

Thompson, P. M., Cornwell, H. J. C., Ross, H. M., and D. Miller. 1992. Serological study of phocine distemper in a population of harbor seals in Scotland. Journal of Wildlife Diseases 28:21-27.

_____, Tollit D. J., Corpe H. M., Reid R. J. and H. M. Ross. 1997. Changes in haematological parameters in relation to prey switching in a wild population of harbour seals. Functional Ecology 11(6): 743-750.

Trites, A. W. and M. A. Bigg. 1992. Changes in body growth of northern fur seals from 1958 to 1974: density effects or changes in the ecosystem? In: Fisheries Oceanography, v. 1 no. 2. 127-136.

Vaughn, G. 1999. Understanding and Evaluating Common Laboratory Tests. Appleton and Lange, Stamford CT. Prentice Hall. 678 p.

Worthy, G. A. J., D. M. Lavigne and W. D. Bowen. 1982. Energy sources in harp seals, *Phoca groenlandica*. In: Perspectives in vertebrate science. The Harp Seal. Eds. D. M. Lavigne, K. Ronald and R. E. A. Stewart. Netherlands.

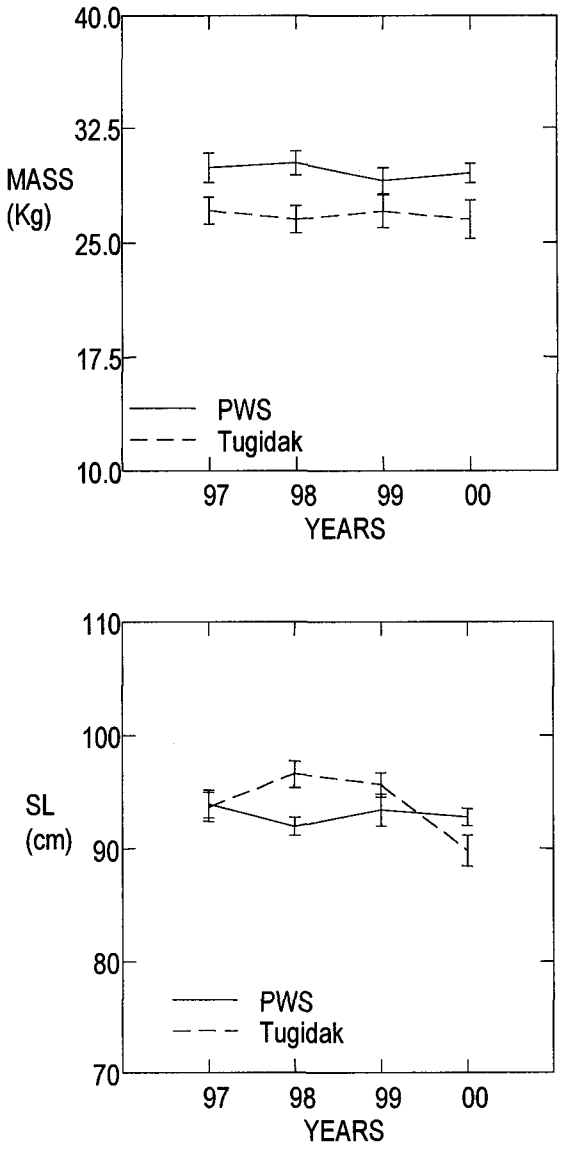

Figure 2.1. Yearly mass and SL values for harbor seal pups from PWS and Tugidak Island, Alaska 1997-2000

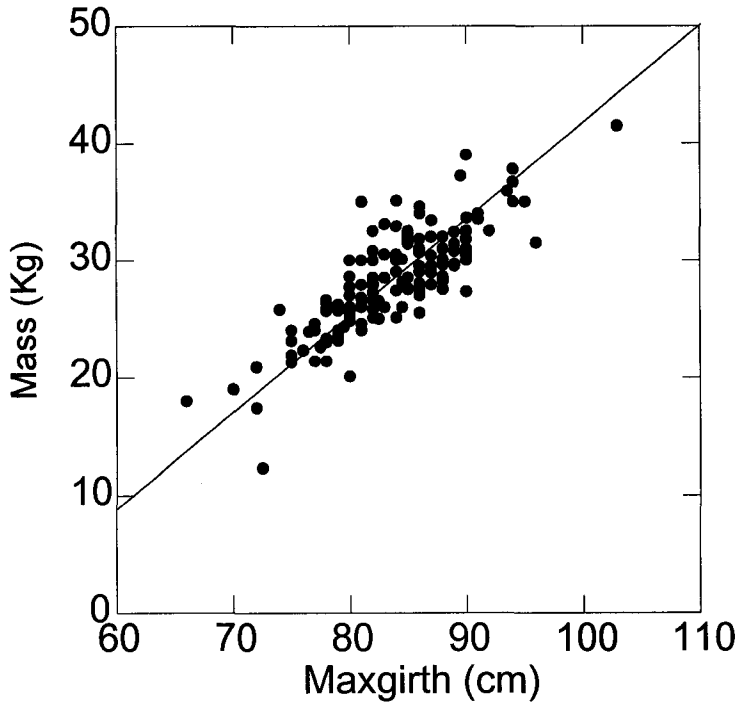

Figure 2.2. Maximum girth to mass regression for harbor seal pups captured from Tugidak Island and PWS Alaska 1997-2000

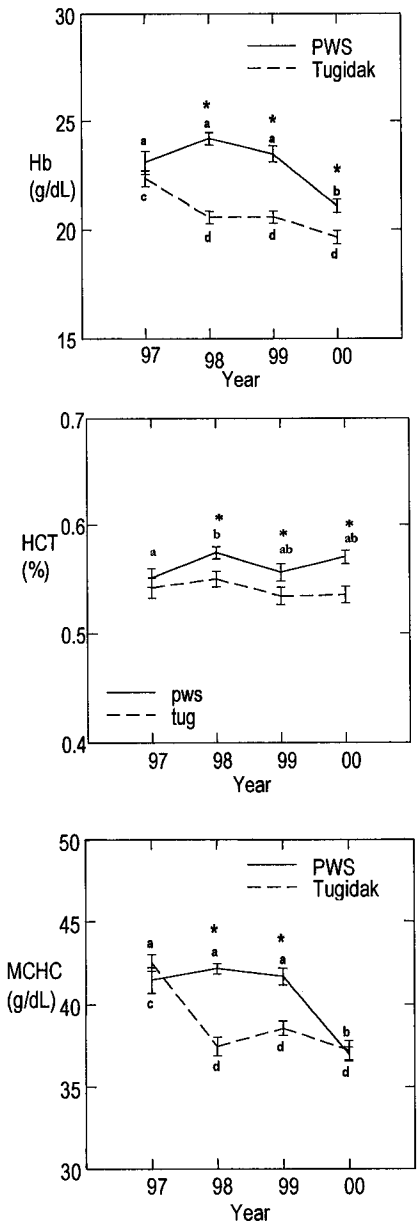

Figure 2.3. Yearly Hct, Hb, and MCHC values for harbor seal pups from Tugidak Island 1997-2000. Note, different letter denotes significant differences within population whereas * among population differences.

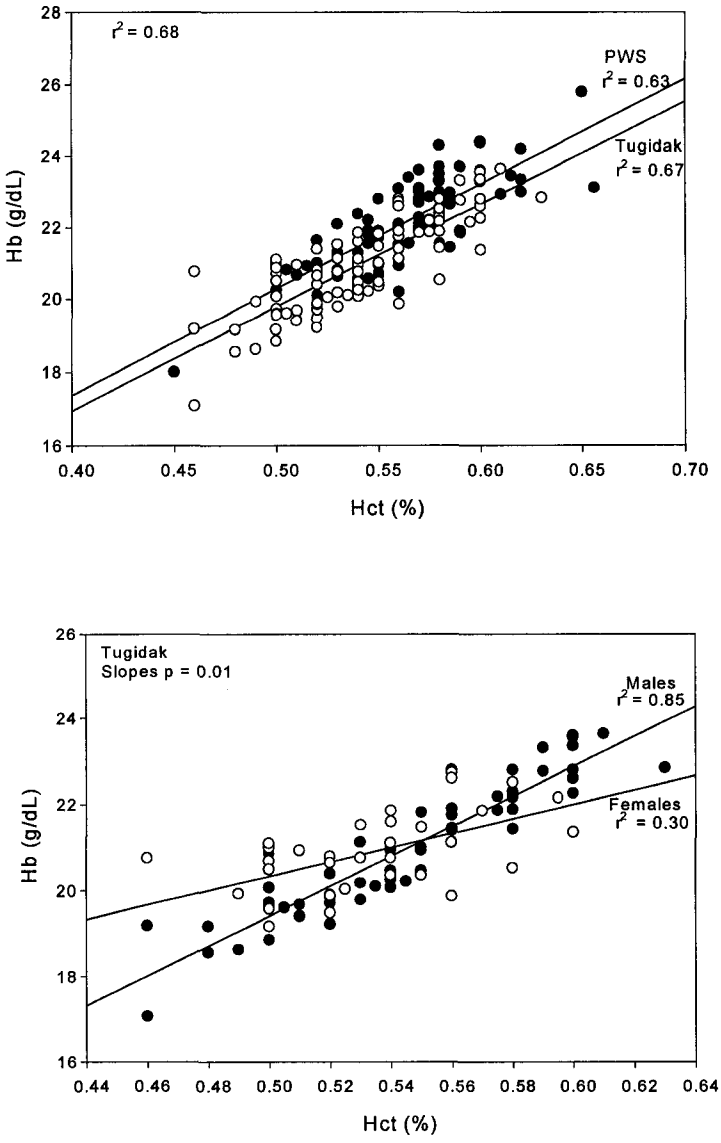

Figure 2.4a,b. Hct and Hb relationships among populations of harbor seal pups from PWS and Tugidak Island, Alaska 1997-2000. Bottom graph gender (males = black circles) differences in Hct/Hb at Tugidak Island. No gender difference was found in regression slopes for PWS pups.

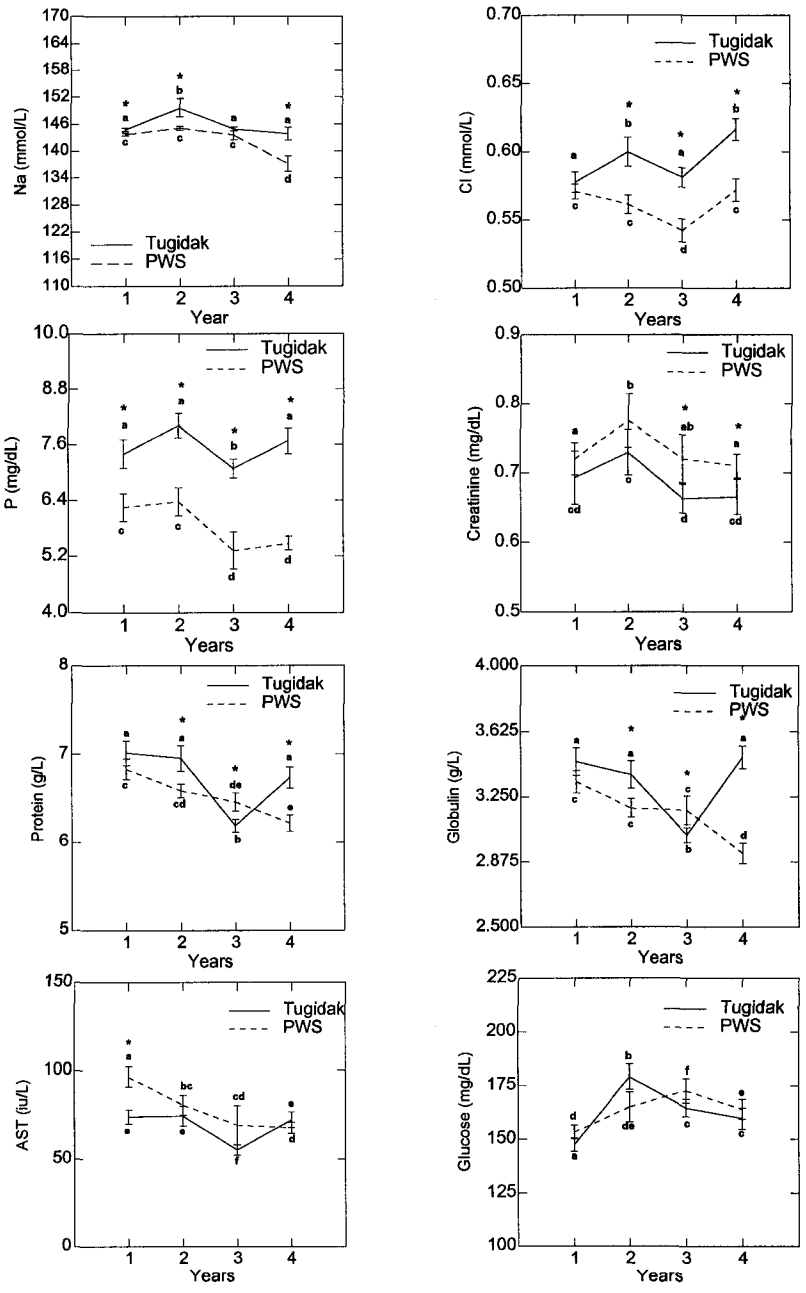

Figure 2.5. Yearly variability of eight blood chemistry parameters in harbor seal

Table 1.1 Morphometric measurements for pups captured within Prince William Sound (PWS) and on Tugidak Island (TUG), Alaska 1997-2000. Note: SL = standard length.

Variable	*n*	*PWS mean*	*PWS SD*	*PWS CV*	*n*	*TUG mean*	*TUG SD*	*TUG CV*	*P value among Sites*	*Pooled N*	*Pooled Mean*	*Pooled SD*	*Pooled CV*
Mass (Kg)*	77	29.8	3.6	0.12	96	26.7	4.9	0.18	0.000	103	28.1	4.6	0.16
SL (cm) [a b c]	77	93.1	4.4	0.05	96	94.1	6.3	0.07	0.541	103	93.6	5.5	0.06
Axial girth (cm)*	77	83.7	5.4	0.07	96	81.4	9.1	0.11	0.034	103	82.4	7.7	0.09
Max girth (cm)	77	84.9	4.9	0.06	69	82.8	5.9	0.07	0.023	148	83.9	5.5	0.07
Hip girth (cm)	77	68.7	6.0	0.09	24	67.0	8.6	0.13	0.278	103	68.3	6.7	0.1
Ax blubber (mm)	29	24.1	3.7	0.15	61	20.8	4.2	0.2	0.000*	90	21.8	4.3	0.2
Mid blubber (mm) [d]	29	25.8	4.5	0.18	67	21.7	3.8	0.17	0.000*	96	22.9	4.4	0.2
Hip blubber (mm) [d]	29	26.5	2.7	0.10	67	21.6	5.5	0.26	0.000*	96	24.1	5.3	0.2

[a] Significant between gender
[b] Significant among years
[c] Significant Y*L interaction
[d] Significant relative blubber thickness (see Results)
* Absolute thickness

Table 1.2. Pearson correlation coefficients for morphometric measurements taken from PWS and Tugidak Is.harbor seal pups between 1997 and 2000. N, n = number sampled, G = girth, and B = Blubber.

PWS	Mass	SL	Ax Girth	Hip Girth	Max Girth	Blubber Axial	Blubber Max	Blubber Hip
n	79	79	79	79	79	29	29	29
Mean	29.2	93.6	83.5	68.3	84.2	23.9	24.9	25.7
SL	0.427							
Ax G	**0.622**	0.214						
Hip G	0.555	0.287	0.352					
Max G	**0.742**	0.255	**0.737**	0.528				
B Axial	0.331	0.419	0.331	0.143	0.275			
B Max	0.272	-0.050	0.211	0.131	0.254	0.429		
B Hip	0.299	0.172	0.369	0.336	0.393	0.410	0.353	

Tugidak	Mass	SL	Ax Girth	Hip Girth	Max Girth	Blubber Axial	Blubber Max	Blubber Hip
n	95	95	95	24	69	62	68	68
Mean	26.9	95.4	81.8	67.0	82.8	21.2	21.5	21.3
SL	0.536							
Ax G	0.439	0.440						
Hip G	**0.681**	0.321	0.419					
Max G	**0.898**	0.460	0.546	**0.685**				
B Axial	0.130	0.162	-0.077	0.170	0.088			
B Max	**0.797**	0.403	0.303	0.573	**0.649**	0.404		
B Hip	0.398	0.252	**0.721**	0.289	0.434	0.091	0.381	

Pooled	Mass	SL	Ax Girth	Hip Girth	Max Girth	Blubber Axial	Blubber Max	Blubber Hip
N	174	174	174	103	148	91	97	97
Mean	28.1	93.6	82.4	68.3	83.9	21.8	22.9	23.0
SL	0.346							
Ax G	0.481	0.259						
Hip G	**0.649**	0.168	0.396					
Max G	**0.836**	0.238	0.580	**0.627**				
B Axial	0.324	0.164	0.103	0.194	0.239			
B Max	0.520	-0.043	0.260	0.355	0.430	0.538		
B Hip	0.440	0.034	**0.644**	0.351	0.450	0.354	0.478	

Table 1.3 Harbor seal pup reference ranges for hematology values and differential leukocytes counts collected at Tugidak Island and within Prince William Sound 1997-2000. Reference range calculated as ± 2SD. Note: n, number; SD, standard deviation; CV, coefficient of variation; PMN, polymorphonuclear cell; WBC, white blood cell; MCHC, mean corpuscular hemoglobin content; NRBC, nucleated red blood cells. [a] arcsine transformed data.[b] Log transformed data. [c] square root transformed data.
[d] Non-normal distribution (Q-Q plot, Kolmolgorov-Smirnoff Probability Test: $p<0.05$), calculated using non-parametric tests
[e] Sample size insufficient for statistical analysis (INS)[f] Significant among Years ($p<0.05$)

Variable	*PWS mean*	*PWS SD*	*PWS CV*	*n*	*TUG mean*	*TUG SD*	*TUG CV*	*n*	*p value among regions*	*Pooled means*			*n*	*Reference Range PWS*	*Reference Range TUG*	*Reference Range Pooled*
										Mean	*SD*	*CV*				
Hematocrit [a]	0.56	0.03	0.06	83	0.54	0.04	0.07	9	**0.000**	0.55	0.04	0.07	175	0.49-0.63	0.47-0.62	0.48-0.63
Hb (g/dL)[b f]	22.1	1.2	0.09	83	20.9	1.3	0.08	9	**0.000**	21.6	2.1	0.09	175	18.4-26.8	17.4-23.9	17.4-25.8
MCHC (g/dL) [f]	40.1	3.4	0.09	83	38.5	3.3	0.08	9	**0.000**	39.3	3.4	0.09	175	33.24-47.0	31.9-45.0	32.4-46.2
PMN (%) [f g]	59.6	12.2	0.20	81	60.7	8.5	0.14	8	0.081	60.2	10.4	0.17	165	35.2-84.0	43.7-77.7	39.3-81.1
WBC (10^6/ml)	9.5	2.6	0.11	62	9.7	3.1	0.09	5	0.709	9.57	2.7	0.1	113	4.3-14.7	3.5-15.9	4.2-15.0
Lymphocyte(%)	29.2	9.2	0.32	81	27.0	10.0	0.37	8	0.559	28.1	9.7	0.34	165	10.8-47.5	6.9-47.1	8.7-47.4
Monocyte (%) [c f]	8.1	5.3	0.66	62	9.6	5.2	0.53	7	**0.043**	8.9	5.3	0.59	140	0-18.8	0-19.9	0-19.5
Eosinophil (%)	4.1	3.6	0.86	65	2.2	1.6	0.70	6	**0.001**	3.2	2.9	0.91	124	0-11.3	0-5.43	0-8.9
Bands (%) [e]	2.5	1.6	0.65	30	1.1	0.4	0.33	7	INS	2.2	1.5	0.69	37	0-4.6	0.36-1.92	0-5.3
Basophils (%) [e]	2.5	0.7	0.28	2	0.9	1.0	1.3	4	INS	0.9	1.1	1.2	43	1.0-4.0	0-2.95	0-3.1
NRBC(/100WBC	2.4	1.1	0.45	12	1.5	3.5	2.4	5	0.469	1.6	3.2	1.94	63	0-3.92	0-8.39	0-8.0

Table 1.4 Harbor seal pup plasma chemistry values for Prince William Sound (PWS) and Tugidak Island (TUG), Alaska from 1997-2000. Reference ranges are ± 2SD; n = 72 for PWS and n = 80 for Tugidak Island. Note: n, number; SD, standard deviation; CV, coefficient of variation. Note: Refer to Methods section for list of acronyms. [a]Log transformed [b]Square root transformed [c]Arc-sine transformed [d] Non-normal, used non-parametric statistics [e]Not transformed, normal distribution [f] Significant among Years [g] Significant among Gender

Variable	*PWS Mean*	*PWS SD*	*PWS CV*	*TUG Mean*	*TUG SD*	*TUG CV*	*P Value*	*Pooled mean values*			*Reference Range PWS*	*Reference Range TUG*	*Reference Range Pooled*
								Mean	*SD*	*CV*			
Sodium (mmol/L) [a f]	140.9	7.5	0.01	148.4	10.4	0.07	**0.001**	144.3	9.1	0.06	125.9-155.9	127.6-169.2	126.1-162.5
Potassium (mmol/L) [a]	3.59	0.67	0.07	3.9	0.4	0.10	**0.001**	3.8	0.3	0.1	2.3-4.9	3.1-4.7	3.2-4.4
Chloride (mmol/L) [a f]	101.8	6.3	0.03	105.1	8.1	0.13	**0.005**	103.7	7.4	0.1	89.2-114.4	88.9-121.3	88.9-118.5
Glucose (mg/dL) [c f g]	162.7	22.6	0.12	168.1	29.1	0.17	0.464	165.9	25.8	0.2	122.7-202.9	109.9-226.2	114.0-217.5
Phosphorus (mg/dL) [d f]	5.68	1.2	0.21	7.6	1.2	0.16	**0.000**	6.71	1.56	0.2	3.3-8.1	5.1-10.0	3.6-9.8
Calcium (mg/dL) [d]	9.67	1.3	0.17	10.0	1.6	0.16	0.097	9.9	1.4	0.2	7.1-12.3	6.8-13.2	7.1-12.7
BUN (mg/dL) [a]	31.3	7.3	0.20	38.3	8.0	0.21	**0.000**	34.9	9.6	0.2	16.7-45.9	22.2-54.4	15.7-54.1
Creatinine (mg/dL) [c f g]	0.68	0.1	0.2	0.75	0.1	0.23	**0.003**	0.7	0.14	0.2	0.5-1.0	0.4-1.0	0.44-1.0
BUN:Creatinine [c]	43.3	11	0.25	55.3	14.4	0.26	**0.001**	49.9	15.6	0.3	21.3-65.3	26.6-84.1	18.6-81.6
Cholesterol (mg/dL)	344.9	98.7	0.27	320.3	69.7	0.22	0.357	337.2	85.3	0.2	147.6-542.4	180.9-459.6	166.6-507.8
Bilirubin (mg/dL) [a]	0.5	0.2	0.4	0.8	1.0	1.5	0.175	0.6	0.7	1.4	0.2-0.9	0-3.0	0-2.15
Total Protein (g/L) [a f]	6.43	0.56	0.06	6.8	1.04	0.15	**0.003**	6.6	0.8	0.1	5.3-7.6	4.8-8.9	5.0-8.2
Globulin (g/L) [f]	3.1	0.42	0.11	3.3	0.6	0.19	**0.002**	3.2	0.5	0.1	2.3-3.9	2.0-4.5	2.2-4.2
Albumin (g/L) [a]	33.3	2.87	0.05	34.9	5.3	0.15	0.066	33.9	4.1	0.1	27.5-39.0	24.2-45.5	25.7-42.1
Albumin:Globulin [c]	1.1	0.16	0.12	1.09	0.4	0.37	0.466	1.07	0.3	0.3	0.8-1.4	0.3-1.9	0.5-1.6
AP (iu/L) [b]	294.7	164	0.58	428.0	185.8	0.43	**0.000**	359.4	178.4	0.5	0-623.5	56.4-799.6	2.6-716.2
AST (iu/L) [b f]	72.3	21.1	0.34	69.7	27.3	0.39	0.194	75.2	29.9	0.4	30.1-114.5	15.0-124.4	15.4-135
ALT (iu/L) [d]	30.0	10.8	0.28	18.7	9.6	0.51	**0.000**	24.6	11.7	0.5	8.4-51.6	0-37.9	1.2-48.0
CPK (iu/L) [a]	798.7	917	0.82	598.7	727.8	0.68	0.140	659.2	815.8	0.9	0-2434	0-2254	0-2290
GGT (iu/L) [a]	18.1	7.0	0.38	20.2	15.9	0.79	0.938	19.3	13.1	0.7	4.1-32.1	0-52.0	0-45.5
LDH (iu/L) [a]	3216	711	0.24	3286	1480	0.45	0.619	3345	1258	0.4	1794-4638	326-6246	829-5861

3

METABOLIC IDENTITY AND ASSESSING HEALTH OF ANIMAL POPULATIONS: A CASE STUDY INVOLVING HARBOR SEALS

ABSTRACT

A current hypothesis for the decline of harbor seals in the Gulf of Alaska is that a shift in prey may have decreased the nutritional quality, causing physiological stress, especially during periods of increased metabolic need. The objectives of this study were to model a suite of blood chemistry /hematology values and to define the metabolic identity of a population of animals. Once a group of blood values is described, the study aimed to define blood values from individual animals that would be considered "outliers" and define population health of distinct groups of harbor seals. This study revealed that plasma chemistry values could be used to discriminate between populations of harbor seal pups. The primary benefit of this technique is the ability to determine environmental stressors and the genetic capacity of the animal to respond to metabolic demands. No evidence of nutritional stress was found for harbor seal pups in a declining population in Prince William Sound versus an increasing population near Kodiak, Alaska.

INTRODUCTION

The National Marine Fisheries Service has defined three provisional management stocks of the Alaska harbor seals (Hill et al. 1997). These stocks are based primarily on haul-out site location, movement data, and trend counts (NMFS Stock Assessment Report 1998, Small 2001). However, harbor seals in Alaska are distributed almost continuously throughout their range, making it difficult to discern where population subdivisions lie and thus identify population boundaries. Making it more confusing, genetic studies have described as many as 18 distinct harbor seal stocks that have sharply declined over the past 30 years Alaska (O' Corry-Crowe 1998). While reasons for these declines have not been substantiated, one current hypothesis is that a shift in prey quality may have decreased the nutritional status of harbor seals, causing physiological stress, especially during periods of increased metabolic need (Alaska Sea Grant 1993).

Unfortunately, trend counts or genetic techniques are not sensitive enough to separate populations based on physiological stress. Instead, an index that can distinguish how groups of animals will respond or adapt to stressors is necessary. There is research demonstrating the significance of using blood chemistry as an index of health or nutritional status in wild marine mammal populations (Geraci and Smith 1975, Castellini et al. 1993, Fadely 1997, Thompson et al. 1997, Trumble and Castellini 2002). Specifically, blood chemistry and hematological

values appear to be sensitive to environmental variation, which may permit changes in physiological status to be examined (Fadely 1997). However, these studies have most often compared blood chemistry values between individuals from different regions or from previously reported clinical reference ranges and interpreted health accordingly. Further, blood chemistry values can be co-dependent, and can vary within populations, among seasons, with year and with gender, all of which add complexity to interpretation (Fadely 1997, Trumble and Castellini 2002). There is a need to understand population level differentiation in plasma chemistry values and thus assess the health of animals occupying the outlier regions of populations, since these regions are often suggestive of poor health (Rebar and Boon 1983, Vaughn 1999).

The objectives of this study are threefold: 1. Describe an integrated suite of blood chemistry /hematology values that can be used to define the metabolic identity of a population of animals. By metabolic identity we mean the metabolic state of the animal that reflects both environmental stressors and the genetic capacity of the animal to respond to metabolic/energetic demands. 2. Once a group of blood values is described, to define blood values from individual animals that would be considered "outliers". 3. To use the metabolic identity and outlier theory to define population health of distinct groups of harbor seals.

METHODS

Blood and subsequent plasma chemistry values were obtained from three populations of wild harbor seal pups; one in California (Fanshell Beach, 36^0 30' N, 121^0 56' W) and two in Alaska, Prince William Sound and Tugidak Island (56^0 30' N, 154^0 40' W), Alaska. Pups were aged between an estimated (based on mass) 2-5 weeks. Newborn pups (with umbilicus) were not sampled.

Blood and morphology collection

Blood was collected from harbor seal pups from California during April-June 1997–2000 whereas samples from Alaska were collected during June/July 1997-2000. Blood collection, capture and handling methods of harbor seal pups captured within Prince William Sound and at Tugidak Island Alaska have been previously described in Trumble and Castellini (2002). Briefly, blood samples were drawn from the extradural intervertebral vein using 18G X 3.5 inch (1.2 X 90mm) spinal needles into heparinized, EDTA, or plain evacuated blood collection tubes (Vacutainer®). Blood samples were chilled until their return to ship or lab (<5h). Whole blood was centrifuged (3000 rpm for 10 minutes) and the plasma or serum separated and stored at -196°C (liquid nitrogen dry shipper). Plasma samples were sent to Fairbanks Memorial Hospital (FMH) for assessment of clinical blood chemistries including sodium (Na), potassium (K), chloride (Cl), calcium (Ca), phosphate (P), cholesterol (CHOL), glucose (GLU), protein (TP), blood urea

nitrogen (BUN), albumin (ALB), creatinine (CR), BUN/creatinine ratio (B:C), globulin (GLOB), albumin/globulin ratio (A:G), bilirubin (BIL), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine phosphokinase (CK), gamma glutamyl transferase (GGT), and alkaline phosphatase (AP). All assays were performed by FMH technicians using automated machine analysis (Kodak Ektachem 550 Analyzer). Meris Laboratories Incorporated and IDEXX veterinary services performed the plasma chemistry analysis for the harbor seals captured in California. The veterinary staff at the Marine Mammal Center in Sausalito, California, performed all hematology for pups captured in California.

Mass was measured using an electronic load cell (± 0.5 Kg, Ohaus I-20W) for all seals. Girth measurements were collected at three locations (maximum, axial, and hip) for seals captured in Alaska, and axial girth measurements were done for pups captured in California. Standard length (SL; straight-line distance between tip of nose and tip of tail) was measured using a tape measure (± 1 cm) with the seal positioned dorsal side up (Castellini and Kooyman 1990, Castellini and Calkins 1993). For pups in Alaska, blubber thickness was measured at three locations (dorsally) at each girth measurement location using a portable ultrasonic unit (Gales and Burton 1987, Scanoprobe II, Model 7310, Scanco, Inc.).

Analysis

Discriminant analysis was used to determine if seal populations could be separated based on plasma chemistry values in the two populations of harbor seal pups in Alaska and one in California. A singular value decomposition of 21 standardized blood parameters was used to produce a set of coordinates in a 3 dimensional space (SPSS, SYSTAT®). Stepwise regressions were used to describe the relationship between morphological measurements and plasma chemistry values. Outliers were defined using multivariate statistical methods and graphical display based on the set of coordinates from plasma chemistry values. Only samples from Alaska were used in outlier comparison. We identified outliers by plotting the vector coordinates from each individual seal in 3 dimensions and labeled as a percentage of distance from the population mean outward. By continuous rotation of the data cloud on all three axes, the relative position of suspected outliers or clusters of outliers in relation to "normal" individuals could be identified. Euclidean similarity cluster analysis was used to define possible groupings (subgroups) of outliers from each population. Subgroups were evaluated for commonality in blood clinical profiles.

Results from seals identified as outliers were removed from the data set and blood chemistry reference ranges were subsequently recalculated for the remaining "normal" population. Blood values from outlier pups were then singly re-plotted against the "normal" reference range values and graphically displayed using

Pearson correlation dissimilarity cluster analysis with complete linkage. Chemistry values obtained from each outlier pup were then assessed using clinical blood chemistry profiles relating to specific physiological systems (Bossart and Dierauf 1990, Franzmann 1985,Vaughn 1999; Stress: AST, ALT, GGT, CK, LDH; Nutrition: A:G, TP, ALB, GLB, BUN, B:C, K, GLU; Electrolyte balance: Na, Cl, K, GLU, CR, Ca and Development: BIL, AP, P. All possible clinical blood profile combinations were generated for each animal identified as an outlier (e.g. seals could be placed into more than one profile group depending on the specific blood chemistry parameters that were outside of the reference range).

RESULTS

A total of 181 plasma samples from individual harbor seals pups were collected from three locations (Tugidak Island, n = 80, PWS, n = 72; California N = 29) There was a significant difference in pup mass among populations (PWS, 29.7 Kg $\pm$ 3.6; Tugidak, 27.0 $\pm$ 4.8, CA, 15.8 $\pm$ 7.4, $P < 0.001$). However, no gender-based differences were detected within population for all morphological measurements. The data set involving harbor seal pups from the California population were not used in the outlier analysis.

Metabolic Identity

Mean plasma chemistry values for the three populations of harbor seal pups are presented in Table 3.1. Using discriminant analysis on standardized plasma chemistry values, the three populations of harbor seals could be separated (Wilkes Lambda 0.142, $F = 13.26$, $df = 20$, $P < 0.001$) and 90% correctly classified (Jackknifed classification matrix, Fig. 3.1, Table 3.2). Using mass as a covariate, 87% were correctly classified. There was less separation between the Alaska pup populations when compared to California (Tugidak and PWS, $F = 10.80$; Tugidak and CA, $F = 13.64$; PWS and CA, $F = 18.30$; $P < 0.001$). Forward stepwise discriminant analysis indicated seven plasma variables accounted for approximately 90% of the total variability among the three populations. These variables include albumin (8%), ALT (11%), phosphorus (8%) creatinine (8%), cholesterol (6%), sodium and chloride. Sodium and chloride attributed more than 50% towards discriminating among populations.

Outliers

A 3-dimensional model of blood chemistry data revealed 12.5% ($n = 9$) of harbor seal pups captured within Prince William Sound and 8% ($n = 6$) of the pups sampled from Tugidak Island were outside the 95^{th} percentile (Fig. 3.2; Fig. 3.3). Hierarchical cluster analysis of individuals classified as outliers revealed PWS produced 4 subgroups and 3 Tugidak Island subgroups (Fig. 3.4a,b). Cluster

analysis of standardized plasma parameters from outlier pups plotted against normal population reference ranges revealed atypical blood parameters of the 15 individuals (Fig. 3.5). Relative percentage of "Nutrition" based blood chemistry profiles was similar among locations yielding 6.5% of Tugidak Island and 7% of PWS pups (Fig. 3.6). Plasma chemistry data from Tugidak Island pups revealed increases in relative percentage of "Development" related profiles when compared to PWS pups, whereas PWS had greater percentages of "Stress" and "Electrolyte" profiles (Fig.3.6).

A stepwise regression was used to describe the relationship between mass, lengths, girths and blubber thickness in Alaska harbor seal pups. This relationship was used as a predictor of body condition. Using 73 samples for which all morphological measures were obtained we estimated:

$$\text{Mass} = 0.691\ (\text{maximum girth}) + 0.231\ (\text{mid blubber thickness}) - 43.591 \qquad (1)$$

($r^2 = 0.74$, $P < 0.05$). A stepwise regression was used to describe the relationship between body condition (the standardized residual deviation from the regression line to Eq. 1) and plasma chemistry variables. Alkaline phosphatase (AP) was the best predictor of body condition in PWS seal pups:

$$\text{PWS Body Condition} = \text{AP}\ (-0.282) + 0.643 \qquad (2)$$

($r^2 = 0.36$), whereas LDH was the best predictor for pups on Tugidak Island:

$$\text{Tugidak Body Condition} = \text{LDH } (-0.473) + 2.543 \qquad (3)$$

($r^2 = 0.29$).

DISCUSSION

Harbor seal pups in each of the three groups tested were distinguished by blood chemistry values. We believe this is the first reported example of plasma chemistry values used as a discrimination technique among marine mammal populations. This technique involves the mathematical grouping of different blood metabolites into an "Identity" which can fingerprint the population or study group. We believe results describe the integrated metabolic translation of genetic makeup and how it responds to environmental input. The significance of this technique is that it uses parameters that are known to respond to environmental cues and health status. Thus, this technique presents a picture of how the animals have translated their genetic makeup into a metabolic response. Also, our technique has revealed that blood chemistry, while a valuable clinical tool for evaluating health and pathological condition in free-ranging and captive marine mammals (Castellini et al. 1993, Thompson et al. 1997, Trumble and Castellini 2002) is population

specific. This would prove useful when interpreting health status between populations.

There are a number of statistical methods that detect outliers within multivariate datasets; however, they usually don't explain why the indicated points are atypical. The identification of outliers hidden in data sets is extremely important, and while there are several methods with which to detect data vectors suspected to be atypical (Billor et al. 2000), these methods are based on significance testing, which needs data that meet the assumption of normality. A common problem in using blood chemistry profiles to define health or nutritional status arises from the statistical probability of a "normal" individual falling outside the reference range in the absence of any condition of clinical significance (5%). It has been reported that, as the number of individual tests using blood parameters increases, the likelihood of a blood parameter or the individual falling out as an outlier increases. For example, on a 20-test panel, the likelihood of abnormal results is extremely high, with the statistical probability of at least one abnormal blood parameter result being slightly more than 64% (Sacher et al. 2000). Our solution uses single data points resulting from integrated blood data vectors accounting for blood parameter interactions, which results in a real graphical representation of the seal based on its blood chemistry (i.e. a metabolic identity). We are not discouraging the use of specific blood parameters to answer specific health questions. However, researchers should be aware of these caveats when

assessing condition. We propose that our method, which follows the method proposed by Bartkowiak and Szustalewicz (1997), enables the visual recognition of the shape of the data cloud and thus can be used to establish varying degrees of separation among individuals or groups. Changing the viewpoint for 3D scatter plots (e.g., simple, spectral, or space plots) may prove to be an effective exploratory technique since it can reveal patterns that are easily obscured unless you look at the "cloud" of data points from an appropriate angle. This can be a major tool in assessing long-term population health. For instance, a shifting in the metabolic identity in long-term blood collection studies can provide clues to changing environmental conditions.

Our results revealed outliers found in PWS pups had a greater percentage of stress-related blood profiles (i.e. AST, CK). Elevated AST and CK can be attributed to muscle damage, increased trauma from capture, or oiled pelage and liver damage in marine mammals (Bossart and Dierauf 1990, Heidel et al. 1996). Interestingly, while we tried to eliminate as many external or confounding variables as possible (Trumble and Castellini 2002), capture techniques between populations differed and may have accounted for the increase in trauma related increases in plasma chemistries in PWS pups. The capture method used in PWS consisted of deploying a large capture net adjacent to a haul-out site where the seals, upon entry in to the water, became entangled underwater until retrieved. Pups were captured on Tugidak Island while hauled-out and usually resting. While

often associated with hepatic damage, we assumed normal hepatic function in captured newborn pups since all liver enzyme values during this study are within previously reported values for free-ranging harbor seals in Alaska (Fadely 1997, Trumble and Castellini 2002). Fadely (1997) reported that there were significant regional and seasonal contributions in ALT and AST in free-ranging Alaska harbor seal plasma chemistries. We do not suspect any liver damage but rather possible dietary differences between the populations. We have found that liver enzyme levels are influenced by diet for captive harbor seals such that diets lower in fat content or higher in protein are associated with increased liver enzyme values (Trumble et al. in prep). It has been reported that ALT was elevated after consuming a high protein diet in vervet monkeys (*Cercopithecus aethiops*) (Johnson et al. 2001). There is supporting evidence from scat and fatty acid analysis, which points to pollock comprising at least 50% of the diet of harbor seals in PWS and Tugidak Island (Small 2001, Iverson et al. 1997). It has been reported that there are structural protein differences between herring and pollock. Therefore, seasonal dietary differences between the populations may account for the differences in liver enzyme or stress levels. Some evidence suggests that even prenatal experiences can have long-term health consequences. In laboratory animals, prenatal or newborn stress has been linked to alterations in adrenocortical and central serotonergic and dopaminergic circuits (Nelson and Bloom, 1997). These observations led to the hypothesis (Barker and Sultan, 1995) that disease

vulnerabilities in newborn and adult life result from "fetal programming" of homeostatic response set points.

During this study we detected a greater percentage of electrolyte-related outlier blood profiles in PWS pups. Measurements of electrolytes have been used to provide information on the water balance of terrestrial mammals, with higher than reference range levels resulting from dehydration (DelGiudice et al. 1987, Rebar and Boon 1983). However, adult seals overcome problems regarding water limitations during lactation by foraging (Boness et al. 1994). Further, it has been reported that dehydration in fasting seals is controlled by the production of metabolic water from fat reserves (Castellini et al. 1993, Crocker and Costa 2001). Our assumption was that electrolyte levels in a pup might reflect the nutritional state of the mother (Schweigert 1993). There is evidence of a positive maternal-fetal health correlation through the transfer of milk constituents or contaminants, especially in primate research. For example, Yang et al. (1997) revealed that the ratio of inorganic to total mercury of milk was significantly higher than that of maternal blood and concluded that the metallic mercury can be transferred to the fetus via the placenta and secreted to a newborn via milk. In northern fur seals (*Callorhinus ursinus*), the transfer of organochlorines from mother to pup via milk can negatively impact the immune function and thus the health of the pup (Beckman 1999). Also, it has been suggested that milk constituents such as sodium, potassium, chloride, water, phosphate, calcium, citrate, iodide, choline,

carnitine, glucose, amino acids and peptides, and fatty acids are transported by the mammary gland and may be mirrored in the blood of the newborn (Shennan and Peaker 2000). Based on our plasma chemistry analysis of outliers, the pup populations appeared to be equally affected by nutritional perturbations. Our study revealed that approximately 7% of each population had atypical nutritional based plasma chemistries, which ultimately led to their outlier status. At this time we cannot determine if this is an ecologically significant percentage of the population.

However, we suggest that this technique may be beneficial in long-term studies where possible shifts in the metabolic state of individuals in the population can be used to assess environmental changes. Plasma electrolyte levels remained within ranges of previously reported values for harbor seals in Alaska (Fadely 1997, Trumble and Castellini 2002). In a recent study involving captive harbor seals, Na^+ and Cl^- values were not influenced by diet but rather with respect to season (Trumble et al. in prep). Seasonal changes in physiology, for example during molt, are genetically determined and may account for the differences in these electrolyte levels. Our study of harbor seal pups suggests that Na^+ and Cl^- accounted for over 50% of the distinction between populations. Since we captured pups during the same season (Trumble and Castellini 2002) we suggest that these electrolytes are genetically determined, thus contributing significantly to the metabolic identity.

Outliers on Tugidak Island had a greater percentage of development-related blood profiles, which include alkaline phosphatase and bilirubin, than pups from PWS. In humans, bilirubin and specific bone growth indicators such as calcium and alkaline phosphatase are elevated in newborns (Vaughn 1999). It has also been reported that bilirubin levels in neonate harbor seals are elevated during the period following birth, with values decreasing with age (Bossart and Dierauf 1990). The occurrence of increased bilirubin values found in Tugidak Island pups may indicate that pups on Tugidak Island were on average developmentally younger. Body mass values would support this since mean mass values were significantly lower in harbor seal pups captured on Tugidak Island (Trumble and Castellini 2002). Levels of alkaline phosphatase also increase with age or development, which we assume to be directly correlated with mass. While apparently sensitive enough to predict developmental status in harbor seal pups, bilirubin or AP did not differ significantly between Alaska populations.

This study suggests that plasma chemistry values can be used to discriminate between populations. The primary benefit of this technique is the ability to determine environmental stressors and the genetic capacity of the animal to respond to metabolic demands. This technique also holds promise for assessing population health. However, it is not without its limitations. Plasma chemistry values should be collected from a large portion of the population in order to define normal population ranges and thus detect outliers. In this study, I sampled

approximately 20 % of the pup population each year. Also, while the outliers in this study were outside of the 95% confidence interval of statistically normal, it must be emphasized that these individuals may represent variations that are unrelated to Alaskan population declines.

ACKNOWLEDGMENTS

This publication is the result of research sponsored by Alaska Sea Grant with funds from the National Oceanic and Atmospheric Administration, Department of Commerce, under grant no. NA 86RG0050 (project no.R/08-08), and from the University of Alaska with funds appropriated by the state. We would like to thank Dr. R. Small for his contributions and assistance throughout the collection phase of this project (Alaska Department of Fish and Game permits #1000 and #358-1585 (NOAA Grant NA87FX0300)). This research was conducted with authorization from the University of Alaska Institutional Animal Care and Use Committee. We also would like to thank K. Frost and her crew of the Alaska Department of Fish and Game for providing blood samples from harbor seal pups from locations within Prince William Sound Alaska between 1997-2000. We would like to acknowledge an Elmer Rasmuson Fisheries Research Center Fellowship for support throughout this project. We would like to thank the following for their assistance in this project, K. Wynne, J.M. Castellini, T. Mau, L. Jemison, J. Jemison, K. Hastings, S. Crowley and R. Daniel.

LITERATURE CITED

Alaska Sea Grant. 1993. Is it Food?: Addressing marine mammal and sea birds declines. Workshop Summary. Alaska Sea Grant Report 93–01.

Barker, D.J.P., and H.Y. Sultan. 1995. Fetal programming of human disease. In M. A. Hanson, J.A.D Spencer and C. H. Rodeck. In:Fetus and Neonate: Physiology and Clinical Applications. Volume 3. 365 pp.

Bartkowiak, A., and A. Szustalewicz. 2000. Outliers: finding and classifying which genuine and which spurious. Computational Statistics, 15:312-320.

_____, and A. Szustalewicz. 1997. Detecting multivariate outliers by a grand tour. Machine Graphics & Vision, 6:487-505.

Beckman, K.B. 1999. Blood organochlorines, immune function and health of free-ranging northern fur seal pups (*Callorhinus ursinus*). Ph.D. Dissertation, University Alaska Fairbanks. 150 p.

Billor, N., Hadi, A.S., and P.F. Velleman.2000. BACON: Blocked Adaptive Computationally-Efficient Outlier Nominators, Computational Statistics & Data Analysis, 34:279-298.

Boness, D.J., D.W. Bowen, and O.T. Oftedal 1994. Evidence from time-depth recorders of a foraging cycle during lactation in a small phocid, the harbor seal. Behavioral and Ecological Sociology, 34:95-104.

Bossart, G.D., and L.A. Dierauf. 1990. Marine mammal clinical laboratory medicine. In: Dierauf, L.A., ed. CRC Handbook of Marine Mammal Medicine: Health, Disease and Rehabilitation. CRC Press, Boca Raton, Florida. pp. 1-52.

Castellini, M.A., and D. Calkins. 1993. Mass estimates using body morphology in Steller sea lions. Marine Mammal Science, 9:48-54.

_____, and G.L. Kooyman. 1990 Length, girth, and mass relationships in Weddell seals (*Leptonychotes weddellii*). Marine Mammal Science, 6:75-77.

_____, Davis R.W., Loughlin T.R. and T.M. Williams. 1993. Blood chemistries and body condition of Steller sea lion pups at Marmot Island, Alaska. Marine Mammal Science, 9:202-208.

Crocker, D.E., and D.P. Costa. 2001. Pinniped Physiology. In: The Encyclopedia of Marine Mammals. Academic Press. New York. pp. 837-842.

DelGiudice, G.D., Mech, L.D., Seal, U.S., and P.D. Karns. 1987. Effects of winter fasting and refeeding on white-tailed deer blood profiles. Journal of Wildlife Management, 51:134-145.

DelGiudice, G.D., L D. Mech, K.E. Kunkel, E.M. Gese, and U.S. Seal. 1992. Seasonal patterns of weight, hematology, and serum characteristics of free-ranging female white-tailed deer in Minnesota. Canadian Journal of Zoology, 70:974-983.

Fadely, B.S. 1997. Investigations of harbor seal (*Phoca vitulina*) health status and body condition in the Gulf of Alaska. Ph.D. Dissertation, Univ. Alaska Fairbanks. 183 pp.

Franzmann, A.W. 1985. Assessment of nutritional status. In: Bioenergetics of wild herbivores. Hudson, R.J. and R.G. White, eds. CRC press. pp 239-259.

Gales, N.J. and H.R Burton. 1987. Ultrasonic measurement of blubber thickness of the southern elephant seal, *Mirounga leonina* (Linn.). Australian Journal of Zoology, 35:207-217.

Geraci, J.R., and T.G. Smith. 1975. Functional hematology of ringed seals (*Phoca hispida*) in the Canadian Arctic. Journal of Fishereis Research Board of Canada, 32:2559-2564.

Heidel, J. R., L. M. Philo, T. F. Albert, C. B. Andreasen, and B.V. Stang. 1996. Serum chemistry of bowhead whales (*Balaena mysticetus*). Journal of Wildlife Diseases, 32:75-79.

Hill, P.S., D. P. De Master, and R.J. Small. 1997. Alaska marine mammal stock assessment 1996. NOAA Technical Memorandum NMFS-AFSC-78. 150 pp.

Huntley, A.C., D.P. Costa, and R.D. Rubin. 1984. The contribution of nasal countercurrent heat exchange to water balance in the northern elephant seal, *Mirounga angustirostris*. Journal of Experimental Biology, 113: 447-454.

Iverson, S.J., K.J. Frost, and L.F. Lowry. 1997. Fatty acids signatures reveal fine scale structure of foraging distribution of harbor seals and their prey in Prince William Sound, Alaska. Marine Ecology Progress Series, 151:255-271.

Johnson, Q., W.J. Veith, and T. Mouton. 2001. The impact of dietary protein intake on serum biochemical and haematological profiles in vervet monkeys. Journal of Medical Primatology, 30(1):61-69.

Nelson, C., and F. Bloom. 1997. Child development and neuroscience. Child Development, 68: 970-987.

O' Corry-Crowe, G.M. 1998. Analysis of genetic and behavioral differences among harbor seal populations in Alaska using microsatellite variation. In: Harbor seal investigations in Alaska, Annual Report. NOAA grant NA57FX0367. Alaska Department of Fish and Game, Anchorage AK. pp. 73-83

Rebar, A.H., and G.D. Boon. 1983. A case-oriented approach to small animal biochemical profiling. Ralston Purina Co. St. Louis, Mo. 104 pp.

Sacher R.A., McPherson R.A., and J.Campos. 2000. Widmann's Clinical Interpretation of Laboratory Tests. 11th ed. F.A. Davis Company, Philadelphia. pp. 10-17.

Schweigert, F.J. 1993. Effects of fasting and lactation on blood chemistry and urine composition in the grey seal (*Halichorus grypus*). Journal of Comparative Biochemistry and Physiology A, 105:353-357.

Shennan, D.B., and M. Peaker. 2000. Transport of milk constituents by the mammary gland. Physiological Reviews, 80: 925-951.

Small, R.J. 2001. Executive Summary *In.* Harbor Seal Investigations, Alaska Department of Fish and Game Annual Report, NOAA # NA87FX0300. Small, R.J. (P.I). pp. 324-344.

Thompson, P.M., Tollit D.J., Corpe H.M., Reid R.J. and H.M. Ross. 1997. Changes in haematological parameters in relation to prey switching in a wild population of harbour seals. Functional Ecology, 11:743-750.

Trumble, S.J. and M.A. Castellini. 2002. Blood chemistry, hematology, and morphology of wild harbor seal pups in Alaska. Journal of Wildlife Management, 66:1197-1207.

Vaughn, G. 1999. Understanding and Evaluating Common Laboratory Tests. Appleton and Lange, Stamford CT. Prentice Hall. 678 p.

Yang, J.M., Jiang Z.Z., Wang Y.L., Qureshi I.A., and X.D.Wu. 1997. Maternal-fetal transfer of metallic mercury via the placenta and milk. Annals of Clinical and Laboratory Science, 27:135-142.

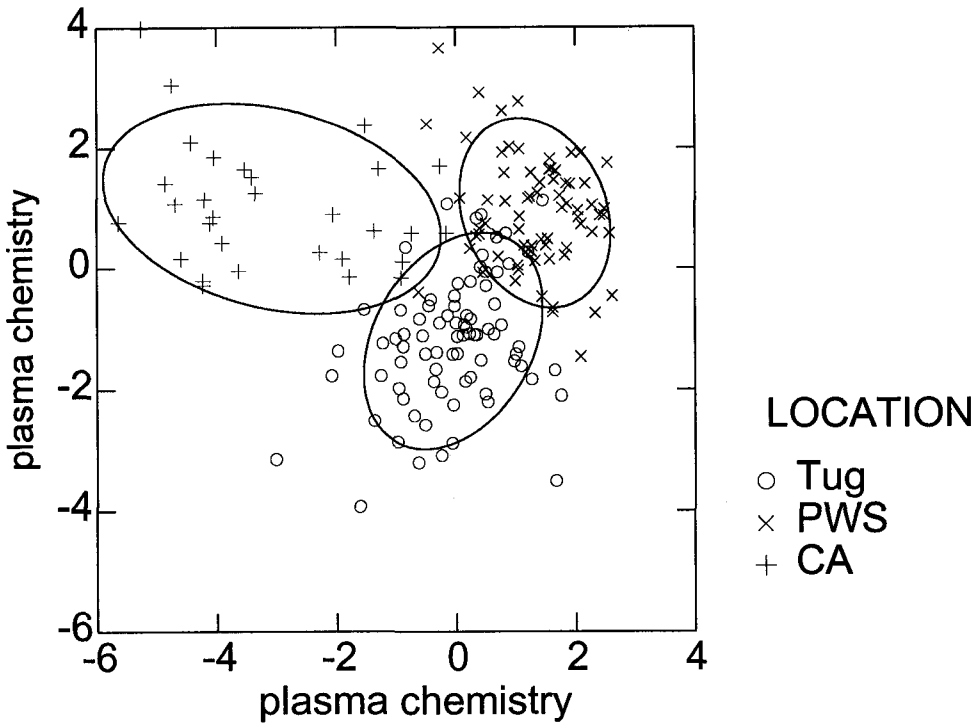

Figure 3.1 Discriminant analysis of standardized plasma chemistry values from 3 harbor seal pup populations; Tugidak Island (Tug) Prince William Sound (PWS) and California (CA). Ellipses encompass 90% of each population

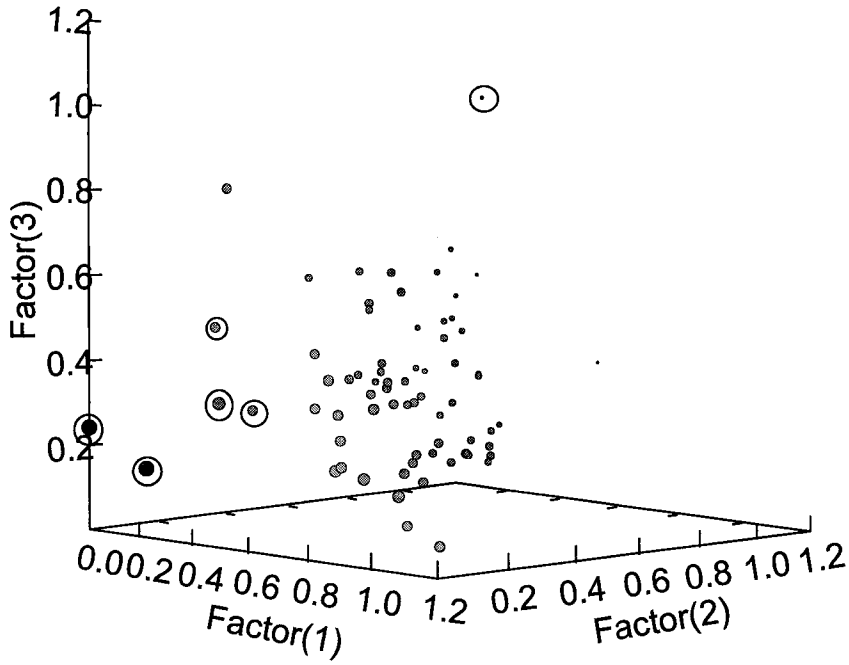

Figure 3.2 3-Dimensional display of the singular decomposition of plasma chemistry values from individual harbor seal pups from Tugidak Island Alaska. Circled values represent pups outside of the 95% confidence interval

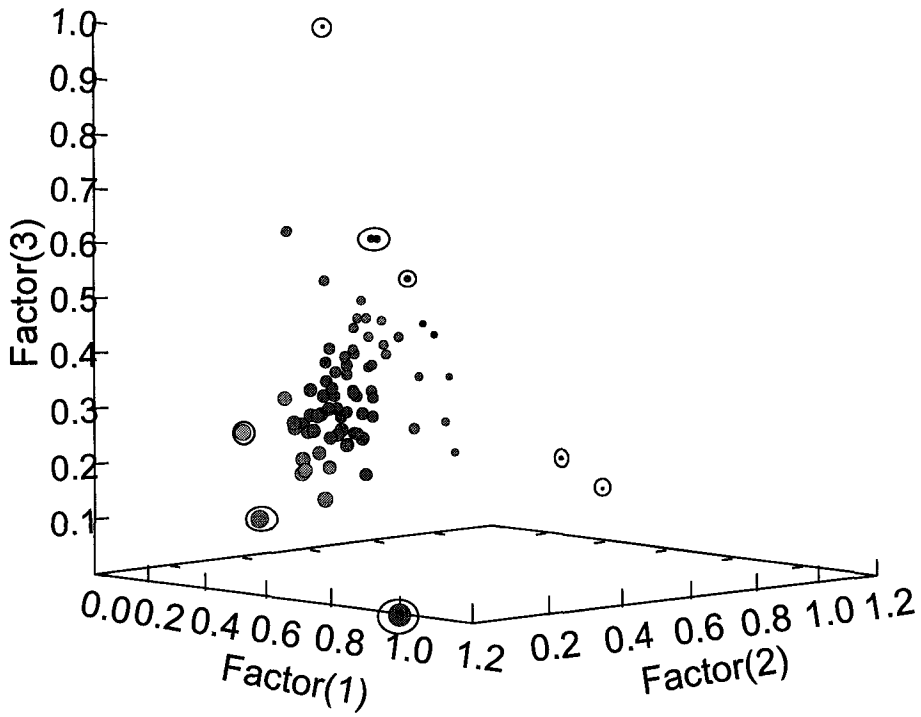

Figure 3.3. 3-Dimensional display of the singular decomposition of plasma chemistry values from individual harbor seal pups from PWS Alaska. Circled values represent pups outside of the 95% confidence interval

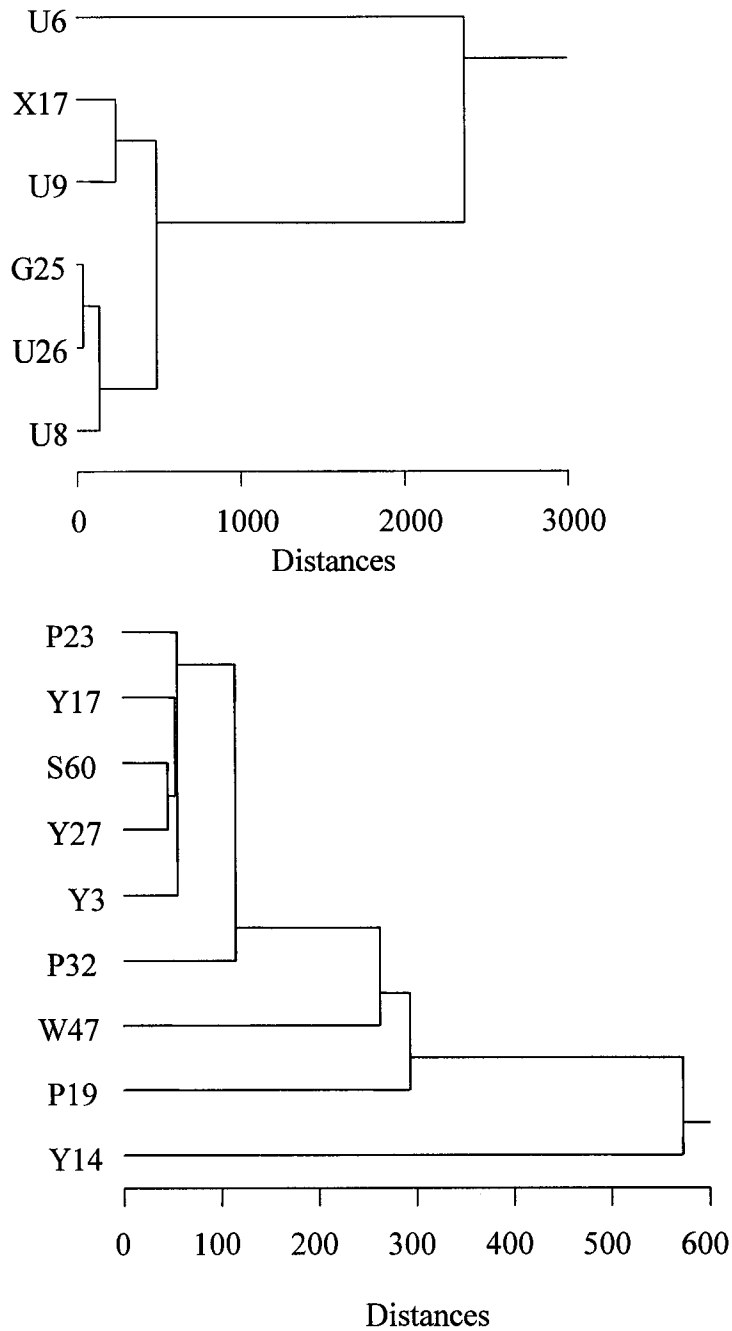

Figure 3.4. Hierarchical cluster analysis of outlier harbor seal pups from Tugidak Is. (a) and PWS (b) captured from 1997-2000. Note: letter represents year. Tugidak Is., 1997 = T, 1998 = U, 1999 = G, 2000 = X; PWS, 1997 = P, 1998 = W. 1999 = S, 2000 = Y) whereas number represents animal identification number

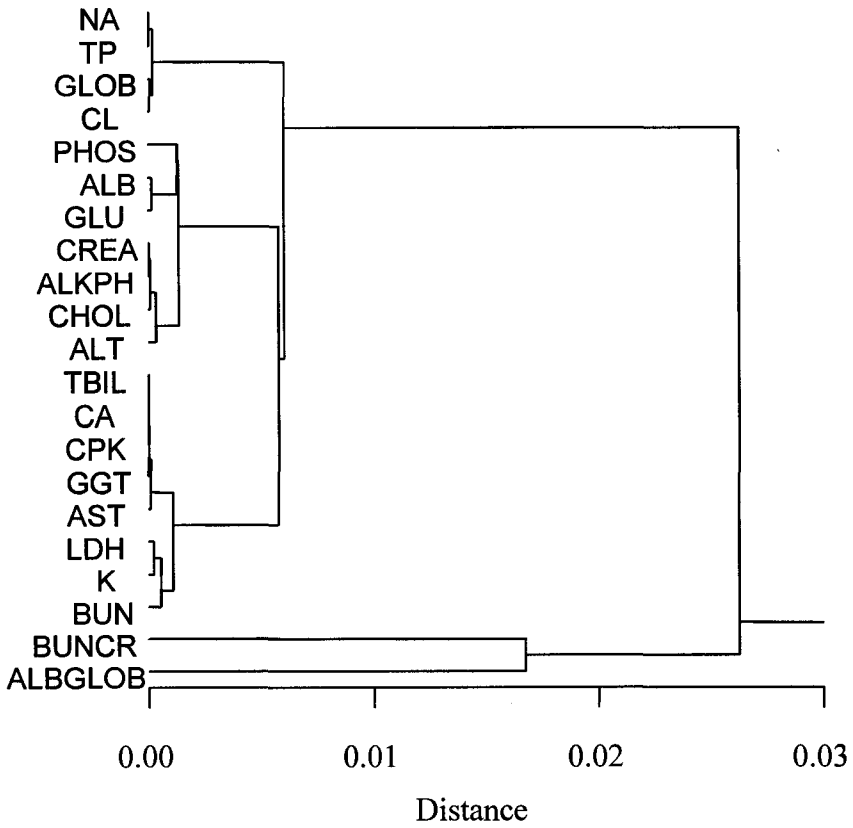

Figure 3.5. Hierarchical cluster analysis of blood chemistry values from one outlier pup (example shown from one Y15) subtracted from population mean values. Note: BUN:creatinine and A:G are identified as blood parameters responsible for pup outlier determination

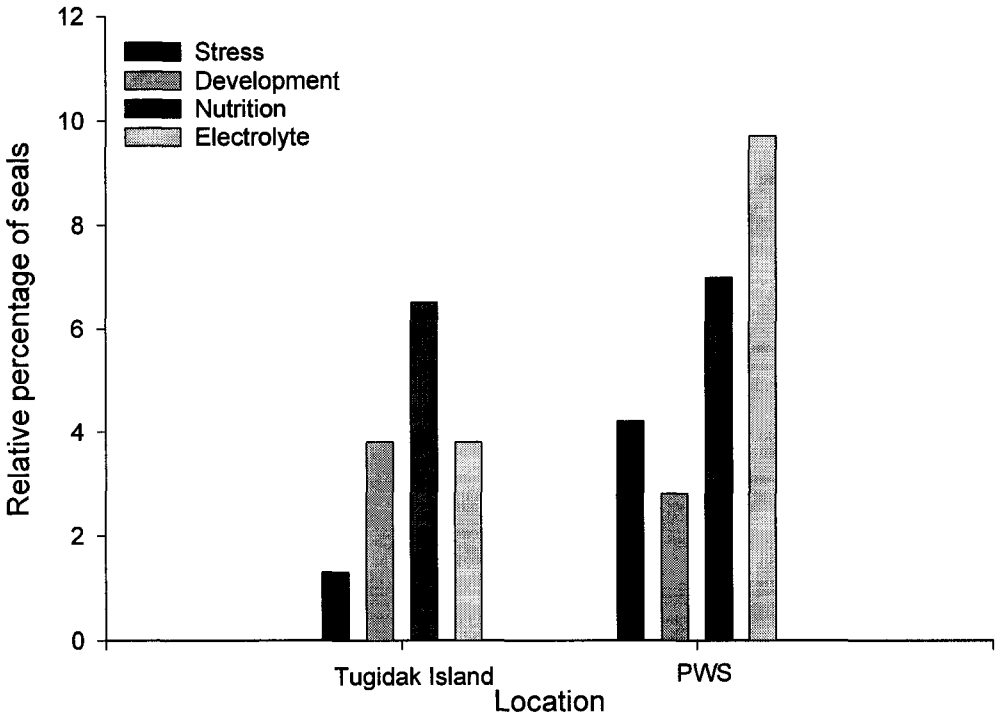

Figure 3.6. Relative percentage of outlier harbor seal pups grouped based on plasma chemistry status

Table 3.1 Mean plasma chemistry ± SD values for 3 populations of harbor seal pups.

Variable	*PWS Mean*	*PWS SD*	*TUG Mean*	*TUG SD*	*Ca mean*	*Ca SD*
Sodium (mmol/L) [a f]	140.9	7.5	148.4	10.4	147.8	5.1
Potassium (mmol/L) [a]	3.59	0.67	3.9	0.4	4.20	0.5
Chloride (mmol/L) [a f]	101.8	6.3	105.1	8.1	100.9	4.8
Glucose (mg/dL) [c f g]	162.7	22.6	168.1	29.1	130.3	27.9
Phosphorus (mg/dL) [d f]	5.68	1.2	7.6	1.2	7.04	1.8
Calcium (mg/dL) [d]	9.67	1.3	10.0	1.6	10.6	1.2
BUN (mg/dL) [a]	31.3	7.3	38.3	8.0	43.3	12.5
Creatinine (mg/dL) [e f g]	0.68	0.1	0.75	0.1	1.0	0.2
BUN:Creatinine [c]	43.3	11	55.3	14.4	43.3	10.4
Cholesterol (mg/dL)	344.9	98.7	320.3	69.7	295.5	78.1
Bilirubin(mg/dL) [a]	0.5	0.2	0.8	1.0	1.6	1.9
Total Protein (g/L) [a f]	6.43	0.56	6.8	1.04	6.9	1.3
Globulin (g/L) [f]	3.1	0.42	3.3	0.6	3.7	1.1
Albumin (g/L) [a]	33.3	2.87	34.9	5.3	32.4	0.4
Albumin:Globulin [c]	1.1	0.2	1.1	0.4	0.9	0.2
Alkaline Phosphatase [b]	294.7	164.4	428.0	185.8	340.0	127.5
AST (iu/L) [b f]	72.3	21.1	69.7	27.3	50.3	24.8
ALT (iu/L) [d]	30.0	10.8	18.7	9.6	22.1	19.2
CPK (iu/L) [a]	798.7	917.8	598.7	727.8	658.7	456.2
GGT (iu/L) [a]	18.1	7.0	20.2	15.9	16.2	11.3
LDH (iu/L) [a]	3216	711	3286	1480	2826	852

Table 3.2. Jackknifed classification matrix for harbor seal pups collected from 1997-2000

	1	2	3	% Correct
Tugidak Is.	69	11	0	86
PWS	3	69	0	96
California	3	2	25	83
Total	75	82	25	90

4

DIETARY AND SEASONAL INFLUENCES ON BLOOD CHEMISTRY, HEMATOLOGY AND MORPHOLOGY IN CAPTIVE HARBOR SEALS[2]

Abstract

Using a repeated measures crossover design we found that of 21 plasma chemistry and 8 hematology variables analyzed in captive harbor seals, 10 changed significantly in response to diet or season over a two-year period. The plasma variables influenced by diet were the liver enzymes ALT, AST and GGT as well as creatinine and the ratio of BUN:creatinine. Sodium, chloride and BUN values changed seasonally. Of the hematology variables tested, no variable changed with diet, and only hematocrit and hemoglobin were influenced by season. There were no between-subject differences in morphology values with regard to season or diet during this project. These data will be of particular importance for the interpretation of plasma chemistry and hematology values measured as a comparative health index in wild populations of seals.

[2] Trumble SJ, Castellini MA, Castellini JM, Mau TL (in prep) Dietary and seasonal influences on blood chemistry, hematology and morphology in captive harbor seals. Physiological and Biochemical Zoology.

Introduction

There is a growing body of research demonstrating the importance of using blood chemistry as an index of health or nutritional status in wild marine mammal populations (Tryland et al. 2002). Specifically, determination of blood chemistry and hematological values that are sensitive to environmental variation permits changes in physiological status to be examined. For example, blood constituents can reflect changes of the intake of protein and energy as well as the degree of fasting (Bossart and Dierauf 1990, de Swart et al. 1995, Rea et al. 1998, Thompson et al. 1997). Increasing our knowledge of circannual metabolic patterns is essential to understanding nutritional and ecological requirements in free-ranging animals. This is an important issue in Alaska, where it has been hypothesized that nutritional stress, due to a shift in prey, has contributed to the decline of many marine mammal species including harbor seals (Phoca vitulina) and Steller sea lions (Eumetopias jubatus) over the past three decades in the Gulf of Alaska (Pitcher 1990, Calkins et al. 1998, Small 2000). However, unlike terrestrial studies where captive animals have been used to establish reference values and examine physiological responses to environmental or nutritional alterations, most studies involving marine mammals report only reference values from free-ranging animals. While such studies provide important reference values for animals in their natural environment, limitations arise when blood values are used as a measure of health and subsequently a comparative index among populations (Trumble and Castellini 2002).

The use of captive marine mammals can provide valuable insight into the relative contribution of external influences on specific physiological responses. However, controlled captive studies involving seasonal blood chemistry changes cannot consider the influence of natural diets, energy expenditure, or the impact of natural perturbations (i.e. ENSO events) (de Swart et al. 1995). Even so, captive studies should be a critical component when assessing dietary or seasonal shifts in metabolic physiology in free-ranging populations. There are many studies reporting on possible seasonal and/or possible dietary influences on blood values in free-ranging pinnipeds (Trumble and Castellini 2002, Rea et al. 1998, Fadely 1997, Castellini et al. 1993, Costa and Ortiz 1982, Schumacher et al. 1992), however, few data exist regarding changes in blood values in captive pinnipeds (de Swart et al. 1995, Ronald et al. 1969). Long-term captive studies involving the effects of diet and season on bloods chemistry values are needed to understand the spatial and temporal physiological responses in free-ranging marine mammals with regard to the environment.

Several studies involving free-ranging marine mammals that have used changes in morphology as an index of body condition (Fadely 1997, Castellini et al. 1993, Calkins et al. 1998). Captive seal studies have also demonstrated that morphology is influenced by diet or season (Rosen and Trites 2000, Ashwell-Erickson and Elsner 1981, Ronald et al. 1969). However, most studies reporting changes in morphology in captive marine mammals have usually been short-term projects. To our knowledge, this represents the first study to focus on the possible combined influences of season and diet on blood chemistry, hematology and morphology in captive harbor seals over long time periods.

The primary objective of this work is to identify the physiological variations expressed as differences in blood biochemistry, hematology values and morphology as it is influenced by seasons and diet quality.

Materials and Methods

Facilities

This study was conducted from August 1998 to September 2000 at the Alaska SeaLife Center (ASLC) in Seward, Alaska under the Marine Mammal Protection Act permit # 881-1143 with approval from the University of Alaska and ASLC Institutional Animal Care and Use Committees. All harbor seals used in this study were housed in either an outdoor pool (350,000 L) or in indoor aquaria used for public display. All morphometric measurements and blood collections were performed every two weeks throughout the experiment. Herring (Clupea pallasi) and walleye pollock (Theragra chalcogramma) were provided from the Alaska commercial fishery (Cook Inlet Processors (pollock) and Icicle Seafoods (herring)) and stored at –20° C. All fish were thawed whole in a water bath for less than 1 h before feeding.

Feeding Protocol

All eight adult harbor seals (mean age 11.8 yrs $\pm$ 8.4) used in this experiment were born in captivity and trained for frequent handling. Except for periods when seals were caged during feeding experiments, seals were subjected to regular seasonal photoperiod and temperature regimes. The husbandry staff at the ASLC fed seals during all feeding trials. There were 6 feeding trials in the following three

periods: September though December (Season 1), January through April (Season 2) and May through August (Season 3). This was repeated in two cycles over 24 months. We fed two groups of three seals an exclusive diet of either herring or pollock while a control group of two seals were fed equal proportions of herring and pollock throughout the study. Seals in groups feeding exclusively on either herring or pollock would alternate throughout the 6 feeding trials so each group fed on either diet 3 times (Figure 4.1). During each feeding session seals were fed to a satiation level that allowed training to proceed.

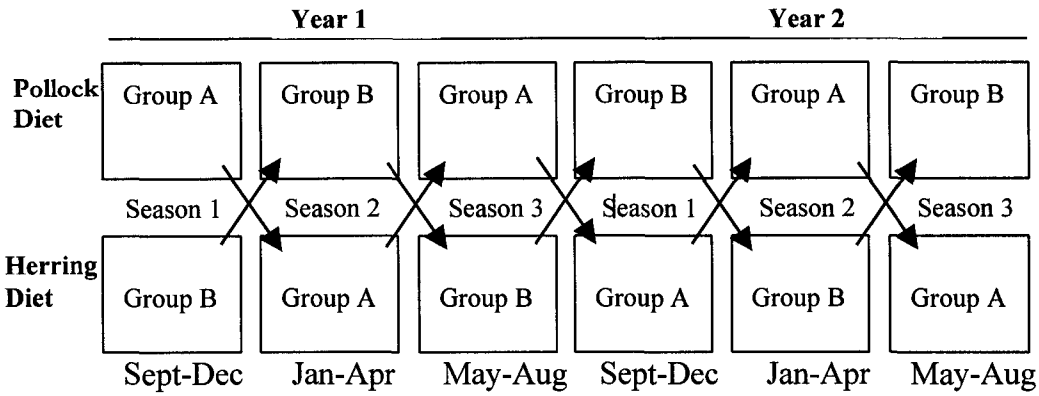

Figure 4.1. Sampling regime depicting the crossover-feeding matrix for captive harbor seals at the ALSC.

This crossover-feeding matrix allowed each group to experience a different diet at similar physiologically relevant times of the year. Group B for example, was fed a herring diet during season 1 in year one and fed a pollock diet during season 1 in year two. Group C (not shown) was fed a 50:50 mix of herring and pollock throughout the experiment.

Individual batches of whole herring and pollock were subsampled (n = 10 for each) periodically (every 1 – 3 months during frozen storage) for analysis. These samples were homogenized in a food grinder and a blender before removing duplicate samples of approximately 10 g. Homogenates of whole fish were frozen at -80°C and then freeze-dried to constant mass under vacuum (VirTis Freeze Dryer Model 5463). Water content was calculated on the basis of the mass lost during lyophilization. Water content was verified by drying separate samples to constant mass in a convection oven at 80°C. Freeze dried samples were used to estimate dry matter crude fat and protein contents. Crude fat (% dry matter) was determined by modified Soxhlet procedure (Model #HT6 Soxtec, Tecator, Foss North America, Silver Spring MD). Nitrogen was determined with an elemental Analyzer (Model # CNS 2000, LECO, St Joseph MI) and expressed as crude protein by assuming that 100g crude protein, contained 16gN (Robbins 1993). Ash content was determined as the dry mass after combustion in a muffle furnace at 500 °C for 12 hours. Carbohydrate content was assumed to be negligible in comparison with lipid, crude protein and water (Sidwell et al. 1974).

Blood collection and analysis. Blood samples were collected from each animal during a post absorptive (fasted $\geq$ 10 hours) state at two-week intervals throughout the experiment. Blood from harbor seals was drawn from the extradural intravertebral vein using 18G X 3.5 inch (1.2 X 90mm) spinal needles into heparinized, EDTA, or serum Vacutainer® blood collection tubes. Collected blood samples were immediately chilled until their analyses (<2h). Plasma samples were processed at the ASLC (IDEXX Ver-

Tex Model 8008 Analyzer) for assessment of "standard" panel of clinical blood chemistries including sodium (Na), potassium (K), chloride (Cl), calcium (Ca), phosphate (P), cholesterol (CHOL), glucose (GLU), protein (TP), blood urea nitrogen (BUN), albumin (ALB), creatinine (Cr), globulin (GLOB), bilirubin (BILI), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine phosphokinase (CK), gammaglobulin transferase (GGT), and alkaline phosphatase (AP). Blood chemistry ratios BUN:creatinine (B:C) and ALB:GLOB (A:G) were also calculated.

We calculated hematocrit (Hct, %) from whole blood using a clinical Hct centrifuge (3 mins @ 11000 rpm). Hemoglobin (Hb) levels were determined by Cyanmethemaglobin spectrophotometric assay methods (SIGMA kit #525). An aliquot of whole blood was used to determine hematology values (QBC® VetAutoread™ Hematology Analyzer) including white blood cells (WBC), granulocytes (Grans), platelets (PLT) and reticulocytes (RET). We calculated mean corpuscular hemoglobin content (MCHC) using Hb values / (Hct x 100). Hct (x 10) and manual red blood cell counts values were used to calculate mean cell volume (MCV).

Morphometrics. Harbor seals were weighed on a Cardinal floor hugger 4'x4' platform scale with Cardinal 210 digital display (0.5 kg resolution), girths at three locations (maximum, axial, and hip) and standard length (SL; straight-line distance between tip of nose and tip of tail) were measured (± 1 cm) with the seal positioned dorsal side up.

Blubber thickness was measured dorsally at three locations at each girth measurement location using a portable ultrasonic unit (Scanoprobe II, Model 7310, Scanco, Inc.).

Statistical analysis. Mean blood chemistry, hematology and morphology longitudinal data were analyzed using repeated-measures analysis of variance (ANCOVA) with diet (herring and pollock) and season (1,2,3) as between-subjects factors and age as a covariate. Between-subject blood values were pooled when non-significant and subsequently analyzed and reported as means for each group. Mauchly's test of sphericity was used on transformed dependent variables. To improve normality, data were transformed such that percentage data were arcsine transformed, alkaline phosphatase and AST values were square root transformed whereas log transformed values included Na, K, Cl, bilirubin, protein, CPK, albumin, GGT, LDH and BUN. To visually examine the possibility that each variable tested exhibited seasonal or dietary patterns, we plotted seasonal and dietary means over time. Points were connected using a cubic spline procedure which joined data points by a series of piecewise cubic polynomials as to form the simplest continuous smoothed curve passing through each point. All statistical tests were performed using SYSTAT (v.10) and SPSS (v.10). P values ≤ 0.05 were considered significant, whereas $0.05 \geq P \leq 0.1$ constituted a trend. All means are reported with $\pm$ SD. Mass data were standardized to the $Kg^{0.75}$ prior to analysis. Blubber thickness relative to body thickness was calculated by dividing the blubber thickness by the body radius at that girth site. Gender effects could not be tested due to inadequate sample size.

Results

Diet Composition

Herring contained more lipid and ash content per dry mass (lipid, x = 48.9% ± 1.8%; ash, x = 3.5% ± 1.4%) than pollock (lipid, x = 21.9% ± 1.7%; ash, x = 7.4% ± 3.3%; $P < 0.001$, N = 80). Pollock contained more water and protein (H_2O, x = 77.0% ± 0.5%; crude protein, x = 67.1% ± 4.3%; herring, H_2O, x = 65.8% ± 0.4%, crude protein, x = 46.6% ± 2.9%; $P < 0.001$) than herring. The mixed diet had water content of 72.1% (± 2.1%) and a mean estimated crude protein value of 52.7% (± 3.4%) while lipid content was 35.3% (± 2.7%). Proximate composition of individual diets (e.g. pollock or herring) fed to harbor seals did not differ over the course of this experiment

Plasma chemistry Out of the 35 blood chemistry, hematology and morphological variables tested in captive harbor seals, 10 (29%) were either influenced by diet or season. Sample sizes used during all statistical analysis are as follows: Herring group (Group A), n = 105; Pollock group (Group B), n = 92; Mixed group (Group C), n = 94; Season 1, n = 54; Season 2, n = 72; Season 3, n = 71.

Dietary influence. Approximately 24% (n = 5) of the 21 blood chemistry values tested were affected by diet (AST, ALT, GGT, B:C, CR) (Figs 4.2 a-c and 4.3 a,b).

Between-subject mean ALT values increased during pollock trials when compared with herring trials for both groups (Group A, mean ALT pollock = 38.9 ±

2.1, mean ALT herring = 29.4 ± 2.5, P = 0.014; Group B, mean ALT pollock = 90.1 ± 4.6, mean ALT herring = 60.7 ± 3.3, P = 0.037, Figure 4.2a).

Mean AST values were also elevated during pollock feeding trials when compared with herring trials (Group A, mean AST pollock = 69.7 ± 4.7, mean AST herring = 48.9 ± 3.8, P = 0.000; Group B, mean AST pollock = 92.1 ± 3.4, mean AST herring = 79.4 ± 4.1, P = 0.002, Figure 4.2b).

Between-subject mean GGT values were elevated during pollock feeding trials when compared with herring trials (Group A, mean GGT pollock = 20.5 ± 5.6, mean GGT herring = 17.4 ± 5.2, P = 0.007; Group B, mean GGT pollock = 20.3 ± 3.3, mean GGT herring = 13.9 ± 4.6, P < 0.000, Figure 4.2c).

Mean creatinine values were elevated during herring feeding trials for both groups (Group A, mean Cr pollock = 1.0 ± 0.03, mean Cr herring = 1.2 ± 0.025, P = 0.007; Group B, mean Cr pollock = 0.8 ± 0.01, mean Cr herring = 0.9 ± 0.02, P = 0.03, Figure 4.3a).

The ratio of B:C was elevated during pollock feeding trials when compared to the herring diet (Group A, mean B:C pollock = 39.1 ± 1.6, mean B:C herring = 32.7 ± 1.2, P = 0.001; Group B, mean B:C pollock = 45.9 ± 1.2, mean B:C herring = 40.5 ± 1.3, P = 0.001, Figure 4.3b).

No significant between-subject effect difference was noticed in mean blood chemistry values with respect to diet for seals in the mixed diet group. However, there was within-subject interaction effect for creatinine values with diet and season in the pollock group (P = 0.05, Figure 4.3a).

Seasonal influence. Of the 21 blood chemistry variables analyzed Na, Cl and BUN (14%) were influenced by season (Figure 4.4).

Mean BUN values were significantly increased during season 3 regardless of diet (Group A, mean BUN season 1 = 36.9 ± 4.9, season 2 = 36.2 ± 3.2, season 3 = 39.5 ± 4.2, P = 0.04; Group B, mean BUN season 1 = 35.4 ± 4.8, season 2 = 32.9 ± 5.0, season 3 = 39.4 ± 6.0, P < 0.000; Group C, mean BUN season 1 = 34.3 ± 3.9, season 2 = 35.4 ± 4.0, season 3 = 38.3 ± 2.9, P = 0.05, Figure 4.4a).

Mean Cl values were also significantly increased during season 1 regardless of diet (Group A, mean Cl season 1 = 113.9 ± 1.3, season 2 = 112.2 ± 1.8, season 3 = 111.9 ± 1.9, P = 0.04; Group B, mean Cl season 1 = 114.1 ± 1.4, season 2 = 112.1 ± 1.1, season 3 = 112.9 ± 1.2, P = 0.01; Group C, mean Cl season 1 = 112.9 ± 1.5, season 2 = 110.9 ± 1.2, season 3 = 110.0 ± 1.2, P = 0.01, Figure 4.4b).

Mean Na values significantly declined during season 3 regardless of diet (Group A, mean Na season 1 = 160.7 ± 2.7, season 2 = 159.5 ± 2.4, season 3 = 156.3 ± 2.9, P < 0.000; Group B, mean Na season 1 = 159.2 ± 2.9, season 2 = 157.2 ± 1.8, season 3 = 153.4 ± 3.1, P < 0.000; Group C, mean Na season 1 = 158.7 ± 3.1, season 2 = 156.9 ± 3.1, season 3 = 154.4 ± 2.9, P = 0.03, Figure 4.4c).

Hematology

Seasonal influence. Of the 8 hematology variables analyzed, only Hct and Hb values were influenced by season (Figure 4.5).

Mean Hct values were significantly increased during season 1 regardless of diet (Group A, mean Hct season 1 = 53.3 $\pm$ 3.6, season 2 = 50.9 $\pm$ 3.2, season 3 = 51.0 $\pm$ 3.1, P = 0.008; Group B, mean Hct season 1 = 56.6 $\pm$ 5.3, season 2 = 51.2 $\pm$ 6.4, season 3 = 52.1 $\pm$ 6.0, P = 0.002; Group C, mean Hct season 1 = 53.7 $\pm$ 3.7, season 2 = 50.8 $\pm$ 5.1, season 3 = 50.7 $\pm$ 4.0, P = 0.02, Figure 4.5a).

Mean Hb values were significantly increased during season 1 regardless of diet (Group A, mean Hb season 1 = 22.7 $\pm$ 1.6, season 2 = 20.4 $\pm$ 2.0, season 3 = 20.5 $\pm$ 1.2, P < 0.000; Group B, mean Hb season 1 = 23.7 $\pm$ 3.0, season 2 = 20.5 $\pm$ 2.7, season 3 = 20.9 $\pm$ 3.0, P < 0.000; Group C, mean Hb season 1 = 22.0 $\pm$ 1.9, season 2 = 20.3 $\pm$ 2.0, season 3 = 20.7 $\pm$ 1.8, P = 0.002, Figure 5.5b).

No dietary influence was found in any hematology variable.

Morphology

While blubber thickness, mass and girth values slightly increased when on the herring diet, there were no between-subject differences with regard to season or diet in any group during this project (Table 4.1).

Discussion

This study demonstrated significant physiological variations in blood biochemistry and hematology in captive harbor seals in response to a change in diet quality (high fat versus low fat) or season.

The herring diet had a 2:1 increase in dry matter fat-to-protein ratio when compared to the pollock diet. While five plasma values were impacted by diet, the only blood chemistry variable that increased during herring intake was creatinine. Creatinine is formed in muscles at a fairly constant rate and enters the circulation only for transportation to the kidneys; therefore, the amount of muscle mass has been considered an important factor on production rate and concentration of creatinine (Rodwell 2000). Also, plasma creatinine concentration has been suggested to increase when protein derived from muscle is used as an energy source at times of nutritional deprivation or after muscle damage, in both cases by increasing the net production of creatinine (Nieminen and Timisjärvi 1983; Jurado and Mattix 1998). Free-ranging white-tailed deer are known to show a positive correlation in their creatinine concentration and total mass (DelGiudice et al. 1992). However, the authors suggested that an increase in creatinine concentration between seasons could be explained by dehydration associated with nutritional deprivation. All seals were fed 2x/d during this study; therefore it is doubtful that these seals experienced nutritional deprivation. Dehydration was also evaluated by measuring Hct, which is known to increase with dehydration in domestic animals (Kaneko 1989) and in deer (DelGiudice et al. 1987). However, there was no correlation between Hct and creatinine for the harbor seals

during this study ($r = 0.18$). Hct values for any seal during this study were not elevated beyond previously published reports for free-ranging or captive harbor seals (Fadely 1997, Bossart and Dierauf 1990). These data suggest that dehydration was an unlikely cause of increasing plasma creatinine concentrations in the captive harbor seals. It has been previously reported that creatinine levels are not influenced by diet in pinnipeds (Bossart and Dierauf 1990). However, this study suggests otherwise. We propose the relative increase of creatinine during the herring diet reflects an overall increase of muscle mass, not associated with an increased intake of lipid but possibly due to differences in dietary protein. Herring protein has been shown to be superior in its ability to promote growth as compared to numerous other species of fish protein (Nilson et al. 1947). During this study lean muscle mass (i.e. D_2O techniques) was not determined on a time scale that could answer this question; therefore, our claim cannot be substantiated. All creatinine values were within previously reported values for harbor seals and the relative differences suggest a dietary influence.

Dry matter crude protein level in pollock was $67.1\% \pm 4.3\%$, whereas in herring it was $46.6\% \pm 2.9\%$. Because fat and protein digestion are used to meet the energetic needs of pinnipeds, the higher levels of protein and lower levels of fat in the pollock diet may have contributed to the increased plasma B:C ratios in captive harbor seals. While few B:C data have been reported in captive harbor seals, similar results have been witnessed in captive cheetahs (Bechert et al. 2002). Creatinine and B:C were influenced with regards to diet changes whereas BUN changed with respect to season with increased levels found during May to August (season 3) in captive harbor seals.

Increased BUN levels are typically associated with starvation/fasting or increased protein intake (Bossart and Dierauf 1990, Castellini and Rea 1992). Elevated BUN levels in this study primarily correspond with molting, which has been reported to extend beyond mid September in free-ranging harbor seals in Alaska (Daniel et al. 2002). During molting, some pinniped species will typically fast as long as 3 months while losing as much as 57% of stored body reserves (Crocker and Costa 2001). Despite maintaining a high level of energy expenditure during this period, seals are able to minimize the depletion of lean body mass, with primary energy reserves coming from adipose tissue. The key adaptation for extended fasting appears to be the ability to spare protein, thereby reducing vital organ damage (Crocker and Costa 2001). This stage of fasting (stage II) is characterized by substantial decreases in BUN levels and urinary excretion of nitrogenous wastes (Castellini and Rea 1992). However, during this captive feeding study the animals were never fasted (Figure 4.6). The increase in pollock intake may indicate a shift in metabolic physiology brought on by increased energetic demands during molt. In these same animals, it was found that because of digestive constraints with increased lipid loads, average dry matter intake of pollock was 50% greater over a herring diet (Trumble et al. 2003). In other words, animals consuming pollock during the molt season could increase intake to meet metabolic demands. A diet high in protein and fat in marine mammals is known to elevate BUN levels (Bossart and Dierauf 1990). It appears that season influences BUN; therefore, caution should be taken when comparing seasonal BUN values.

We detected a dietary influence in plasma concentrations of liver enzymes (ALT, AST and GGT) such that there were relative increases while on a pollock diet when compared to a herring diet. An increased protein intake may result in the additional removal of nitrogen. The dominant reactions involved in removing amino acid nitrogen from the body are transaminations, which funnel nitrogen from free amino acids into compounds producing ammonia, or their amine groups are converted to urea by the urea cycle. Transaminations involve moving an α-amino group from a donor α-amino acid to the keto carbon of an acceptor α-keto acid. These reversible reactions are catalyzed by aminotransferases, such as AST and ALT (Vaughn 1999). AST levels have been implicated in contributing to muscle damage as a result of handling or injury in marine mammals (Heidel et al. 1996, Bossart and Dierauf 1990). These enzymes have also been used as clinical markers to assess tissue damage in animals fed contaminated food or liver disease in obese humans, with increasing plasma or serum levels indicating an increased extent of damage (Fishbein et al. 2003). However, all liver enzyme values during this study are within previously reported values for free-ranging harbor seals in Alaska (Fadely 1997, Trumble and Castellini 2002). Therefore, we do not suspect any liver damage but rather a dietary effect in captive harbor seals. Fadely (1997) reported that there were significant regional and seasonal contributions in ALT and AST to the total variance of individual or environmental factors in free-ranging Alaska harbor seal plasma chemistries. This study suggests that these regional differences are a function of dietary differences between regions.

During this study, plasma concentrations of Na^+ and Cl^- in captive harbor seals were influenced by season, with increased levels found September to December (season 1) for both electrolytes and Na^+ levels also elevated from January to April (season 2). While electrolyte levels remained within ranges of previously reported values (Fadely 1997, Trumble and Castellini 2002), values declined from season 1 (winter) to season 3 (summer). Na^+ and Cl^- are the major mineral elements in the blood and body fluids, and play a major role in regulating body water. Along with Hct, measurements of electrolytes have been used to provide information on the water balance of terrestrial mammals, with higher than reference range levels resulting from dehydration (DelGiudice et al. 1987). There appeared to be similar seasonal trends with Hct and electrolyte values increasing concurrently in captive harbor seals. While positive, the correlation was weak between Hct and Na^+ ($r = 0.10$) or Cl^- ($r = 0.13$) for harbor seals during this study. This would suggest that these values are not influenced by water balance as commonly reported for other mammals (Rietkerk et al. 1994). Previous data on adult harbor seals in Alaska suggest regional and gender-specific differences in mean electrolyte levels (Fadely 1997). It has been reported that in fasting seals dehydration is controlled by the production of metabolic water from fat reserves (Houser and Costa 2003, Crocker and Costa 2001, Castellini et al. 1993). Seals during this study maintained an average intake of approximately 150 g dry matter per $K^{0.75}$ throughout each season (Trumble et al. 2003). This would maintain metabolic water production through the catabolism of fat from diet and blubber reserves. Therefore, dehydration, or hemoconcentration, was an unlikely cause of

increasing electrolyte concentrations in the captive animals. Since diet did not appear to influence electrolyte values, we suggest that the seasonal differences in electrolyte values are metabolic shifts attributable to an endogenous rhythm, particularly photoperiod or temperature-dependent changes which can influence behavior (de Swart et al. 1995). Harbor seals during this study we subjected to natural temperature and photoperiod rhythms (60°10' N., 149°30' W).

Captive harbor seals had peak Hct and Hb values from September to December (Season 1) with the nadir occurring during the period from May to August (Season 3). Circannual patterns in hematological values in captive adult harbor seals are not available for comparison. However, Hct and Hb values for captive adult harp seals did show circannual rhythms (Ronald et al. 1969) whereas seasonal differences were reported in free-ranging harbor seals in Alaska (Fadely 1997). Several terrestrial studies have also reported similar results of exhibiting consistent circannual patterns with Hct/Hb peaks during winter and decreased values during summer (Knick et al. 1993, Seal and Mech 1983). Free-ranging black bears also showed similar rhythms with Hb and Hct decreasing during summer (Franzmann and Schwartz 1988). In terrestrial mammals, these rhythms are usually correlated with poor nutrition periods (DelGiudice et al. 1987). We did not detect any physical or biochemical signs of inadequate nutrition for the captive harbor seals during this study (see previous paragraphs). de Swart et al. (1995) reported longitudinal data in newly weaned captive harbor seal pups and revealed an age effect with regards to Hct and Hb rhythms. There

was no age effect in Hct/Hb levels for the adult captive seals during this study. Harbor seals showed nearly a 10% difference between peak and low Hct values. It is known that during molt harbor seals experience increased haul-out times in order to dry and warm their skin (Frost et al. 1995). Whether the drop in Hct reported in this study is a function of the increased haul out times or a physiological adaptation to molting is unknown at this time.

During this study we observed numerous seasonal variations, which appear to be indicative of shifts in the seals metabolic physiology. Although seasonal metabolic shifts are partially attributable to an endogenous rhythm, the intensity of their expression was most likely affected by nutritional changes and, however small, concomitant alterations of body condition. The blood and hematology data obtained during this study were collected from captive harbor seals and thus should not be representative of "normal" blood values in free-ranging populations. However, we do report blood variables that are influenced by changes in diet quality and seasons. These data will be of particular importance for the interpretation of seasonal or temporal blood chemistry and hematology values measured as a comparative health index in seals between populations.

Acknowledgments

This project was funded in part by the *Exxon Valdez* Oil Spill Trustee Council project # /341 to M.A.C. However, the findings and conclusions presented by the authors are their own and do not necessarily reflect the views of position of the Trustee Council. We would like to acknowledge The Elmer Ramuson Fisheries Research Center Fellowship for their support of S.J. Trumble throughout this study. We would also like to acknowledge the veterinary and husbandry staff at the Alaska SeaLife Center in Seward, Alaska for care and feeding of the animals. We also thank the following for assistance during this project: M. Bando, H. Harmon, L. Petraskas, L. Cornick, S. Harper, and ASLC interns.

Literature Cited

Ashwell-Erickson S, Elsner R (1981) The energy cost of free existence for Bering Sea harbor and spotted seals. In: Hood, D.W. and J.A. Calder (eds.), The Bering Sea Shelf: Oceanography and Resources, Vol. 2., pp.869-899. Univ Wash Press.

Bechert U., J. Mortenson, E.S. Dierenfeld, P. Cheeke, M. Keller, M. Holick, T.C. Chen, and Q. Rogers. 2002. Diet composition and blood values of captive cheetahs (*Acinonyx jubatus*) fed either supplemented meat or commercial food preparations. Journal of Zoology and Wildlife Medicine 33:16-28.

Bossart G.D., and L.A. Dierauf. 1990. Marine mammal clinical laboratory medicine. In: L.A. Dierauf (eds). CRC Handbook of Marine Mammal Medicine: Health, Disease and Rehabilitation. CRC Press, Boca Raton, Florida. Pp. 1-52.

Calkins D.G., E.F. Becker, and K.W. Pitcher. 1998. Reduced body size of female Steller sea lions from a declining population in the Gulf of Alaska. Marine Mammal Science 14(2):232-244.

Castellini M.A., and L.D. Rea. 1992. The biochemistry of natural fasting at its limits. Experientia. 48: 575-582.

Castellini M.A., R.W. Davis, T.R. Loughlin, and T.M. Williams. 1993. Blood chemistries and body condition of Steller sea lion pups at Marmot Island, Alaska. Marine Mammal Science 9(2):202-208.

Costa D.P., and C.L. Ortiz. 1982. Blood chemistry homeostasis during prolonged fasting in the northern elephant seal. American Journal of Physology 242:R591-R595.

Crocker D.E., and D.P. Costa. 2001. Pinniped Physiology. In: The Encyclopedia of Marine Mammals. Academic Press. New York. Pp. 837-842.

Daniel R.G., L.A. Jemison, G.W. Pendleton, and S.M. Crowley. 2003. Molting phenology of harbor seals on Tugidak Island, Alaska. Marine Mammal Science 19(1):128–140.

DelGiudice G.D., L.D. Mech, U.S. Seal, and P.D. Karns. 1987. Effects of winter fasting and refeeding on white-tailed deer blood profiles. Journal of Wildlife Management 51:865–873.

DelGiudice G.D., L.D. Mech, K.E. Kunkel, E.M. Gese, and U.S. Seal. 1992. Seasonal patterns of weight, hematology, and serum characteristics of free-ranging female white-tailed deer in Minnesota. Canadian Journal of Zoology 70:974–983.

de Swart R.L., P.S. Ross, L.J. Vedder, F.B.T.J. Bionk, P.J.H. Reijnders, P.G.H. Mulder, and A. D.M.E. Osterhaus. 1995. Haematology and clinical chemistry values for harbour seals (*Phoca vitulina*) fed environmentally contaminated herring remain within normal ranges. Canadian Journal of Zoology 73: 2035-2043.

Fadely B.S. 1997. Investigations of harbor seal (*Phoca vitulina*) health status and body condition in the Gulf of Alaska. Ph.D. Dissertation Univ. Alaska Fairbanks. 183 pp.

Fishbein M.H., M. Miner, C. Mogren, and J. Chalekson. 2003. The spectrum of fatty liver in obese children and the relationship of serum aminotransferases to the severity of steatosis. Journal of Pediatric Gastroenterology and Nutrition 36(1):54-61.

Franzmann A.W., and C.C. Schwartz. 1988. Evaluating condition of Alaskan black bears with blood profiles. Journal of Wildlife Management 52(1):63-70.

Frost K.J., L.F. Lowry and J. Ver Hoef. 1995. Habitat use, behavior and monitoring of harbor seals in Prince William Sound, Alaska. Annual Rep. For *Exxon Valdez* Oil Spill Restoration Project (Restoration Projects 94064 and 94320-F), Alaska Dept. of Fish and Game, Wildlife Conservation Division, Fairbanks. 87pp.

Heidel J.R., L.M. Philo, T.F. Albert, C.B. Andreasen, and B.V. Stang. 1996. Serum chemistry of bowhead whales (*Balaena mysticetus*). Journal of Wildlife Diseases 32(1):75-79.

Houser D.S., and D. P. Costs. 2003. Entrance into stage III fasting by starveling northern elephant seal pups. Marine Mammal Science: 19(1):186–197.

Jurado R., and H. Mattix. 1998. The decreased serum urea nitrogen-creatinine ratio. Archives of Internal Medicine 158:2509–2511.

Kaneko J.J. 1989. Clinical Biochemistry of Domestic Animals. 4th ed. Academic Press, New York. 106 pp.

Knick S.T., E.C. Hellgren, and U. S. Seal. 1993. Hematologic, biochemical, and endocrine characteristics of bobcats during a prey decline in southeastern Idaho. Canadian Journal of Zoology 71:1448-1453.

Nieminen M., and J. Timisjärvi. 1983. Blood composition of the reindeer. II. Blood chemistry. Rangifer 3:16–32.

Nilson H.W., W.A. Marintek, and B. Jacobs. 1947. Nutritive value for growth of some fish proteins. Commercial Fisheries Review 9:1-7.

Pitcher K.W. 1990. Major decline in the number of harbor seals (*Phoca vitulina richardsi)* on Tugidak Island, Gulf of Alaska. Marine Mammal Science 6(2):121-134.

Rea L.D., M.A. Castellini, B.S. Fadely, and T.R. Loughlin. 1998. Health status of young Alaska Steller sea lion pups as indicated by blood chemistry and hematology. Comparative Biochemistry and Physiology Part A, 120:617-623.

Rietkerk, F.E., E.C. Delima, and S.M. Mubarak. 1994. The hematological profile of the mountain gazelle (*Gazella gazella*): variations wit sex, age, capture method, seasons and anesthesia. Journal of Wildlife Diseases 30:69-76.

Robbins, C.T. 1993. Wildlife nutrition and feeding. Academic Press, San Diego, 2nd edition. 352 pp.

Rodwell V.W. 2000. Conversion of amino acids to specialized products. Pp. 347–358 in R.K. Murray, D.K. Granner, P.A. Mayes, and V.W. Rodwell, eds. Harper's Biochemistry. 25th ed. Appleton & Lange, Stamford, Conn.

Ronald K., M.E. Foster, and E. Johnson. 1969. The harp seal, *Pagophilus groenlandicus* (Erxleben, 1777). II. Physical blood properties. Canadian Journal of Zoology 47:461-468.

Rosen D.A.S., and A.W. Trites. 2000. Digestive efficiency and dry-matter digestibility in Steller sea lions fed herring, pollock, squid, and salmon. Canadian Journal of Zoology 78:234-239.

Schumacher U., G. Rauh, J. Plotz, and U. Welsch. 1992. Basic biochemical data on blood from Antarctic Weddell seals (*Leptonychotes weddelli*): ions, lipids, enzymes, serum proteins and thyroid hormones. Comparative Biochemistry and Physiology 102A (3): 449-451.

Seal U.S., and L.D. Mech. 1983. Blood indicators of seasonal metabolic patterns in captive adult wolves. Journal of Wildlife Management 47(3):704-715.

Sidwell V.D., P.R. Foncannon, N.S. Moore, and J.C. Bonnet 1974. Composition of the edible portion of raw (fresh or frozen) crustaceans, finfish and mollusks. I. Protein, fat, moisture, ash, carbohydrate, energy value, and cholesterol. Marine Fisheries Review 36: 21-35.

Small R.J. 2000. Executive Summary In: Harbor Seal Investigations, Alaska Department of Fish and Game Annual Report, NOAA # NA87FX0300. Small, R.J. (P.I). pp. 324-344.

Thompson P.M., D.J. Tollit, H.M. Corpe, R.J. Reid, and H.M. Ross. 1997. Changes in haematological parameters in relation to prey switching in a wild population of harbour seals. Functional Ecology 11(6):743-750.

Trumble S.J., P.S. Barboza, and M.A. Castellini. 2003. (in press). Digestive constraints on an aquatic carnivore: effects of feeding frequency and prey composition on harbor seals. Journal of Comparative Biochemistry and Physiology Part B.

Trumble S.J. and M.A. Castellini. 2002. Blood chemistry, hematology, and morphology of wild harbor seal pups in Alaska. Journal of Wildlife Management 66(4):1197-1207.

Tryland M., E. Brun, A.E. Derocher, J.M. Arnemo, P. Kierulf, R.A. Olberg, and O. Wiig. 2002. Plasma biochemical values from apparently healthy free-ranging polar bears from Svalbard. Journal of Wildlife Diseases 38(3):566-575.

Vaughn G. 1999. Understanding and Evaluating Common Laboratory Tests. Appleton and Lange, Stamford CT. Prentice Hall. 678 p.

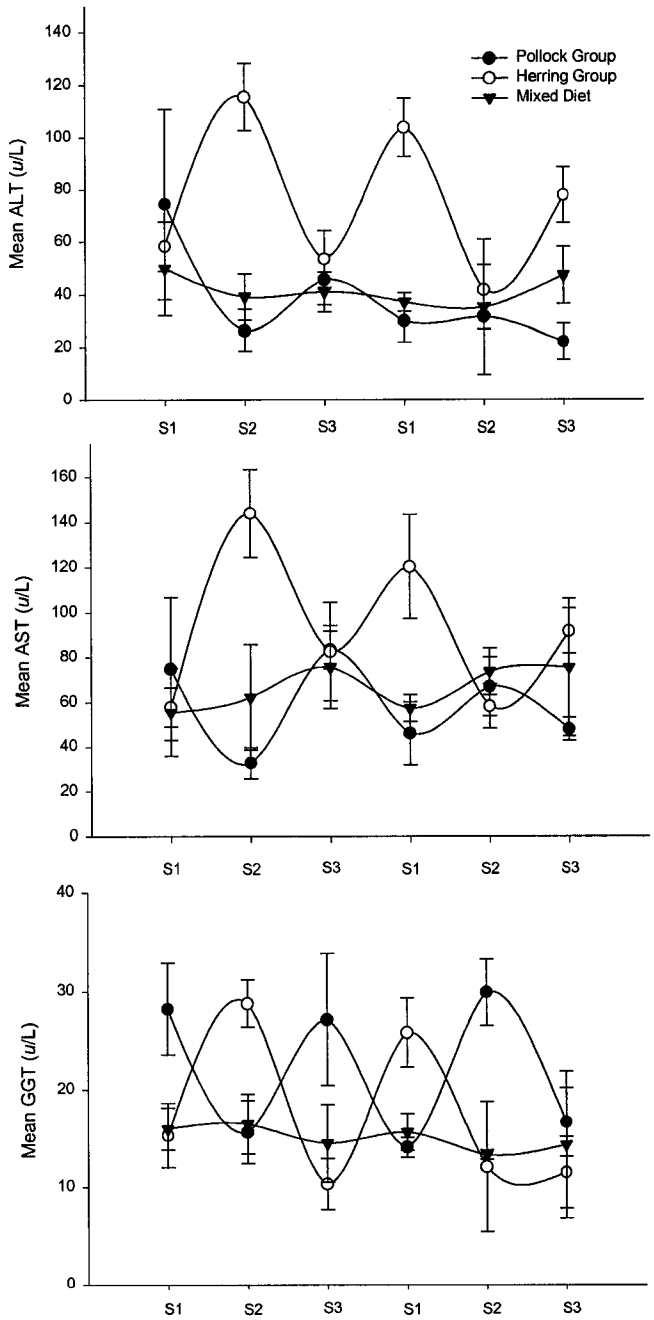

Figure 4.2. Mean seasonal ± SD ALT (a), AST (b) and GGT (c) levels for captive harbor seals fed pollock, herring or a mixed diet at the ASLC between August 1998 and September 2000.

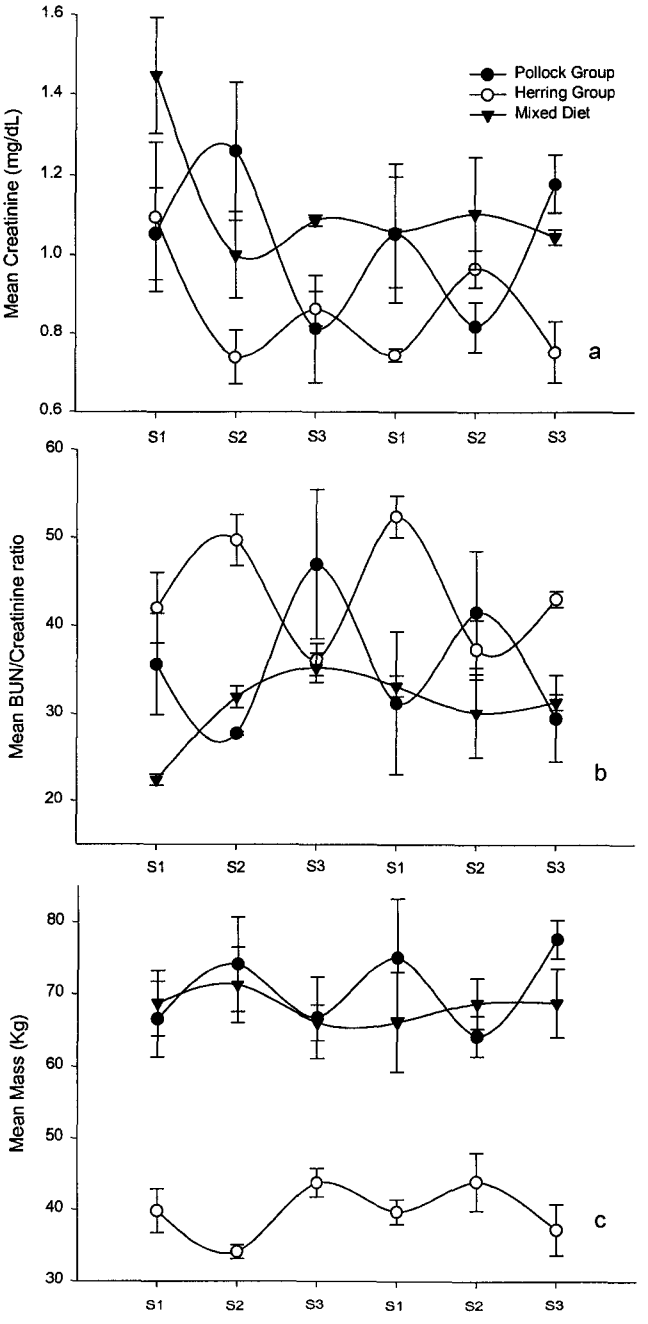

Figure 4.3. Mean seasonal ± SD creatinine (a), B:C (b) and mass (c) levels for captive harbor seals fed pollock, herring or a mixed diet at the ASLC between August 1998 and September 2000.

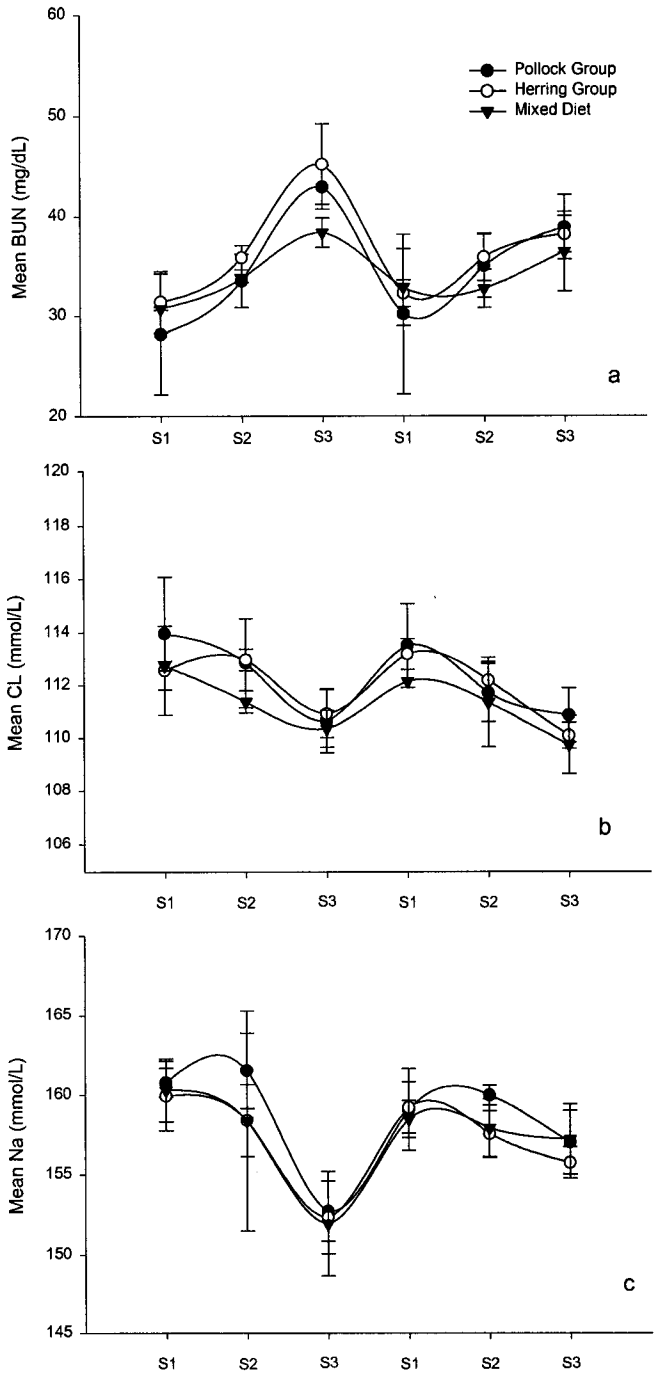

Figure 4.4. Mean seasonal ± SD BUN (a), Cl (b) and Na (c) levels for captive harbor seals fed pollock, herring or a mixed diet at the ASLC between August 1998 and September 2000.

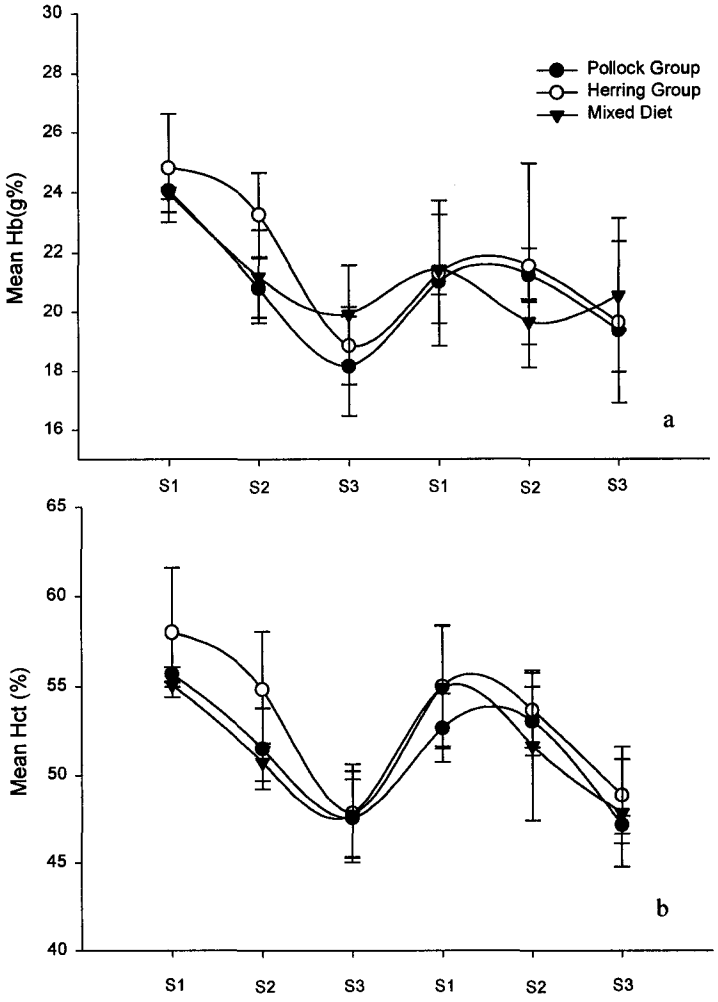

Figure 4.5. Mean seasonal ± SD Hb (a) and Hct (b) levels for captive harbor seals fed pollock, herring or a mixed diet at the ASLC between August 1998 and September 2000.

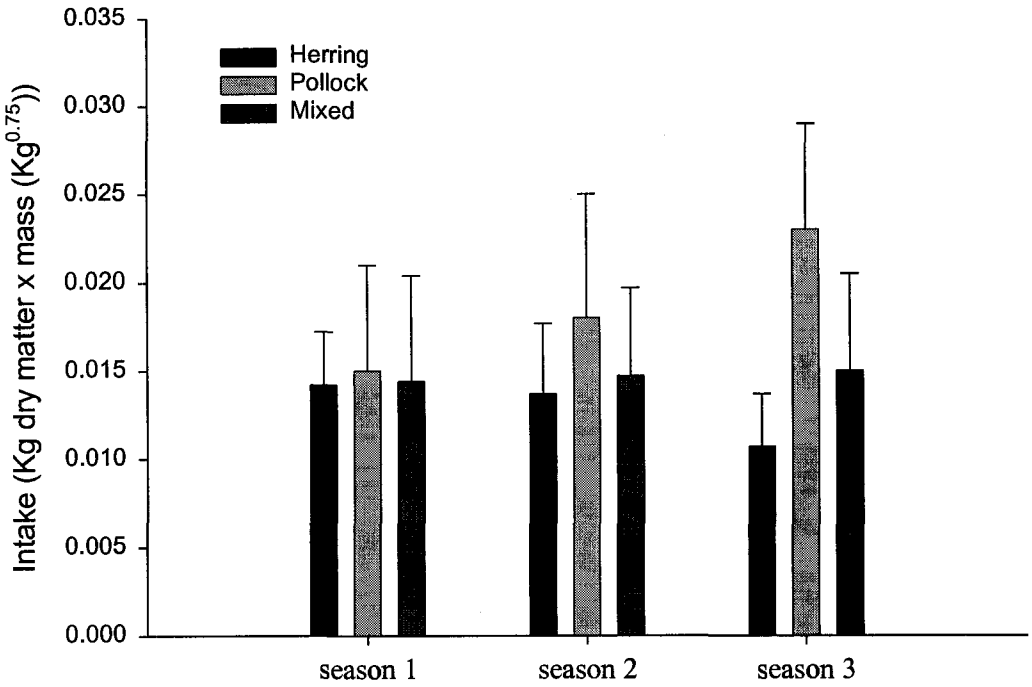

Figure 4.6. Mean seasonal $\pm$ SD intake of herring and pollock for captive harbor seals.

Table 4.1. Overall morphometric measurements for captive harbor seals during feeding trials at the Alaska SeaLife Center from September 1998 to August 2000.

		Pollock		*Herring*		*Mixed*		*Seasons*					
Variable	***n***	***mean***	***SD***	***Mean***	***SD***	***mean***	***SD***	***1***	***SD***	***2***	***SD***	***3***	***SD***
Mass (Kg)	71	52.1	8.6	57.5	7.5	54.8	8.0	53.	7.7	55.6	9.1	55.7	1.5
SL (cm)	71	130.6	6.0	130.5	11.5	129.7	11.4	125.	16.5	131.4	9.2	132.4	7.8
Axial girth (cm)	5[illegible]	93.4	8.6	98.5	5.9	95.7	7.9	93.	1.8	96.2	6.2	97.6	5.7
Ax blubber (mm)	51	15.3	3.0	17.9	3.6	16.6	3.3	17.5	1.9	16.5	3.9	16.8	3.5
Mid blubber (mm)	51	17.4	3.6	20.6	4.5	19.0	4.1	17.	3.4	17.6	3.9	18.2	4.1
Hip blubber (mm)	51	18.9	3.5	20.9	4.7	19.7	4.4	19.7	4.7	19.4	4.6	20.0	3.9

5

DIGESTIVE CONSTRAINTS ON AN AQUATIC CARNIVORE: EFFECTS OF FEEDING FREQUENCY AND PREY COMPOSITION ON HARBOR SEALS[3]

Abstract

We hypothesized that increased feeding frequency in captive harbor seals would increase nutrient loads and thus reduce retention time and the digestive efficiency of natural prey. We measured daily feed intake and excretion during 6 feeding trials and fed herring (49% lipid), pollock (22% lipid) or an equal mix of each diet over 24 months. Animals were accustomed to feeding at either high (high FF) of low (low FF) frequency. Body mass and intake did not vary with season. Although mean retention times were similar between diets and feeding frequencies, solute and particulate digesta markers separated at high feeding frequency. Consistent dry matter digestibility resulted in greater gut fill from pollock than from herring. Digestible energy intakes from pollock were approximately 25% greater than from either herring or the mixed diet. Lipid digestibility of herring declined from 90% to 50% when lipid intake exceeded 60 g $kg^{-0.75}$ d^{-1}. Our hypothesis of a trade-off between intake and digestion was not supported for protein but was supported for lipid. Results of this

---

[3] S.J. Trumble, P.S. Barboza and M.A. Castellini (in press). Digestive constraints on an aquatic carnivore: effects of feeding frequency and prey composition on harbor seals. Journal of Comparative Physiology B.

study imply that a flexible digestive system for harbor seals can compensate for ingesting prey of lower energy density by increasing gut fill and enhancing protein and lipid assimilation, to sustain digestible energy intake.

Introduction

The flexibility of the digestive system to accommodate and process nutrients may determine food selection and the nutritional niche occupied by an animal (Perrin 1994). Mammalian carnivores consume a wide variety of vertebrate and invertebrate prey to meet the demands of their diverse life histories, body sizes and body plans (Carbone et al. 1999). High-energy requirements of large terrestrial carnivores (>25Kg, Carbone et al. 1999) are associated with a preference for large prey (Biswas and Sankar 2002, Paltridge 2002). Consequently, terrestrial carnivores spend a great deal of foraging effort pursuing larger prey when hunting alone (Caro 1994). Large aquatic carnivores such as pinnipeds, must contend with patchily distributed food sources, and long periods of searching interspersed with short periods of food consumption. It has been suggested that the metabolic rates among marine mammal carnivores are greater than terrestrial carnivores and that a comparatively larger alimentary tract is required for supporting the increased energetic demands of an aquatic lifestyle (Williams et al. 2001). However, variation in rates of energy and nutrient intake also depends on the quality, size and species of available prey (Kastelein et al. 1990).

Aquatic carnivores must process large amounts of lipid and protein in each feeding bout. Large meals require large capacity in the foregut with a concomitant ability to produce digestive secretions and absorb the products of degradation (Stevens and Hume 1995). Although endogenous enzymes can degrade the substrates from vertebrate prey, net uptake from the digestive tract can be limited by demand for secretions and by the time required for digestion and absorption (Jumars and Martinez del Rio 1999; Penry and Jumars 1987). As a result, digestive efficiency may be reduced by high rates of digesta flow due to high rates of intake or low capacity of the digestive tract (Sibly and Calow 1986). Digestive efficiencies in pinnipeds fed fish range from 83% to 95% (Lawson et al. 1997; Rosen and Trites 2000a), with higher digestibility values for prey rich in lipids (Ashwell-Erickson and Elsner 1981; Fadely et al. 1990; Fisher et al. 1992; Keiver et al. 1984; Rosen and Trites 2000a). The relationship between digestion and intake is of particular interest in declining populations of pinnipeds because prey quality could theoretically limit the animals' ability to meet requirements for maintenance (Rosen and Trites 2000b). Consequently, a nutritional deficiency resulting from a shift in prey, where small fatty fishes were replaced with low fat fish such as pollock has been a leading hypothesis concerning the decline in many aquatic bird and mammal populations in Alaska (Alverson 1992). Alverson (1992) suggests that this change, while possibly linked to the content of energy or fatty acids in the fish, may be also a function of the schooling behavior and the ease of capture of that prey. Subsequent studies have reported that condition indices in an aquatic mammal, the Steller sea lion (Eumetopias jubatus), indicate that

individuals from a declining population may be suffering from a nutritional deficiency (Alverson 1992, Calkins et al. 1998). Additionally, studies of captive Steller sea lions have suggested that this pinniped is unable to maintain body mass on a diet consisting exclusively of walleye pollock (Theragra chalcogramma) when compared to a more lipid dense fish such as Pacific herring (Clupea pallasii) (Rosen and Trites 2000b). Those responses to diets that are low in lipid may reflect low intakes or a constraint to their digestion and absorption.

In this study, we examined changes in digestive efficiency that accompanied changes in nutrient loads in captive harbor seals (Phoca vitulina). We hypothesized that increased feeding frequency would increase nutrient loads on seals and thus reduce retention time and the digestive efficiency of natural prey. We fed two prey items (herring and pollock) of different lipid and protein composition in two frequencies to simulate discontinuous feeding (once daily, low feeding frequency, low FF) or frequent feeding (four times each day, high FF) during winter, summer and autumn. Digestive performance was measured as voluntary food intake along with digesta flow of particulate and solute markers, and apparent efficiency of assimilating dry matter, lipid and nitrogenous compounds.

Methods

This study was conducted from August 1998 to September 2000 at the Alaska SeaLife Center (ASLC) in Seward, Alaska under permit # 881-1143 to M. Castellini for the

Marine Mammal Protection Act, and with approvals from the Institutional Animal Care and Use Committees at University of Alaska Fairbanks and at ASLC. All harbor seals were born and raised in captivity and routinely housed in either an outdoor pool (approx. 350,000 L) or in indoor aquaria used for public display. Seals were weighed (± 0.5 kg) every two weeks throughout the experiment. Herring and pollock were provided from the Alaska commercial fishery (pollock from Cook Inlet Processors, and herring from Icicle Seafoods) and stored at –20° C. All fish were thawed whole in a water bath for less than 1 h before feeding.

Experimental Design

Animals were accustomed to frequent handling and to cages used for collection of excreta. During the feeding trials, eight seals (mean age 11.8 yrs ± 8.4) were housed in cages with a 12:12 h light regime and ambient temperatures ranging from 7°-15°C. Two sizes of cage with mesh floors (8cm^2) were used: adult seals were held in cages 2.2 m long x 1.1 m wide x 1.5 m high while a smaller cage (1.8 m long x 0.9 m wide x 1.2 m high) was used for sub-adults. Body mass was recorded (± 0.5 Kg) before and after each period in cages. All seals were periodically hosed with fresh water. Seals were fed fish by hand until food was rejected during each trial to determine maximum voluntary intake. All feces were collected every two hours during the cage experiment for up to 72 hours. All excreta eliminated were collected onto aluminum trays suspended beneath each cage and positioned to allow urine to be separated from feces. We measured daily feed intake (mass of all fish presented minus the mass rejected or

regurgitated) and excretion during collection periods of 48-72 h. We conducted 6 feeding trials over 24 months in two annual cycles as follows: September to December, January to April and May to August.

Feeding Treatments

We fed 2 groups of 3 seals an exclusive diet of either herring or pollock while a control group of 2 seals were fed equal proportions of herring and pollock throughout the study. Each diet was fed for approximately 3 months prior to caged feeding trials. Feeding frequency was manipulated to simulate discontinuous feeding. Captive seals on the low FF diet were fed once per day during morning hours whereas seals on the high FF diet were given four (~25% of daily intake per feeding) feedings spaced equally over a 12-hour period. All feeding frequency experiments were started 10 days prior to caging. Animals were fed to satiety on each presentation of food. During the high FF trials, a seal did not eat at least three times per day on only two experiments. Subsequent exclusion of those data did not affect mean intakes, digestibilities and digest flow parameters or their statistical comparison.

Diet analysis

Pollock averaged 164 mm in length (Standard Length, SL), while herring averaged 94 mm (SL). We cut pollock to approximate the size of whole herring to remove any bias of prey size on intake. A multivitamin was administered once per day, usually with the first feeding. Digesta passage was measured with single oral doses of chromic oxide

(Cr_2O_3, 250 mg) and cobalt-ethlyene-diamine-tetra-acetic acid (Co-EDTA, 250 mg) to mark the solid and liquid phases of the digesta, respectively (Robbins 1993). Co-EDTA was produced as a lithium salt following the procedure of Uden et al. (1980). Both markers were administered as a ground powder placed in gelatin capsules and positioned in either the opercular cavity or the lateral muscles of the fish during the first morning meal of the feeding trial. Seals were observed to ensure all doses were consumed.

Individual batches of whole herring and pollock were sampled for analysis every 1 – 3 months during frozen storage (n = 10 per species). Samples of fish were homogenized in a food grinder and a blender before removing duplicate sub samples of 10 g for analysis. Homogenates of whole fish were frozen at -80°C and then freeze-dried to constant mass (VirTis Freeze Dryer Model 5463). Water content was calculated on the basis of the mass lost during lyophilization and verified by drying separate samples to constant mass in a convection oven at 80°C. Freeze dried samples were used to measure concentrations of crude fat and crude protein in dry matter. Crude fat was determined by extraction with petroleum ether in a modified Soxhlet procedure (Model #HT6 Soxtec, Tecator, Foss North America, Silver Spring MD). Nitrogen (N) was determined with an elemental Analyzer (Model # CNS 2000, LECO, St Joseph MI) and expressed as crude protein by assuming that 100 g crude protein contained 16 gN (Robbins 1993). Ash content was determined as the dry mass after combustion in a muffle furnace at 500 C for 12 h. Carbohydrate content of fish was assumed to be

negligible in comparison with lipid, crude protein and water (Sidwell et al. 1974). Energy content of dry matter was therefore estimated from crude fat and protein content with calorific values for fat (39.3 $kJ.g^{-1}$) and protein (24 $kJ.g^{-1}$; Blaxter 1989).

Chemical Analysis

Fecal samples collected from each animal during a single experimental period were combined (as a proportion of the total fecal mass) to provide a representative subsample of the feeding trial. Discrete samples of feces were collected from each seal prior to caging for baseline concentrations of markers. We assumed that the midpoint of each collection period was the time of defecation. Discrete samples of feces were collected into plastic bags and stored at –20°C for analysis. Fecal dry matter content was determined by freeze-drying samples to constant mass. Dried feces were ground to a fine powder with mortar and pestle and a mill (Wiley Mill no. 20 mesh). Lipid, N, and ash content of feces were determined by the same methods used for analyzing fish. All results are expressed as wet weight (wwt) and percent dry matter (DM). Assays for Chromium (Cr) and Cobalt (Co) markers were performed by digestion in a mixture of 70% v/v HNO_3 (1000 ml), 32 M H_2SO_4 (200 ml), 70% v/v $HCLO_4$ (343 ml) and deionized water (57 ml). Digestions were performed at 165°C for 15 min followed by 315°C for 35 min. Duplicate digests were diluted with distilled, deionized water and assayed by atomic absorption spectrometry (Model 5000, Perkins Elmer, Norwalk, CN).

Statistics and calculations

Mean retention time (MRT) was calculated as:

$$MRT = \frac{\sum_{i=1}^{u} t_i c_i \cdot \Delta t_i}{\sum_{i=1}^{u} c_i \Delta t_i}$$

where c_i is the concentration of the marker in the $_i$th sample, collected at time t_i, over the time interval Δti (Warner 1981). Symmetry of marker pulses was measured by pairwise comparison of the delay between T_{50} and T_5, and the time between T_{95} and T_{50}, where T_{50} is time at maximum excretion, T_5 is time at 5% excretion, T_{95} is time at 95% excretion, and T_{end} is time at final excretion of marker.

Apparent digestibility (%AD) was calculated for dry matter, lipid, crude protein, and ash in food and feces (Robbins 1993) as follows:

$$\text{Apparent Digestibility (\%)} = \frac{\text{Food intake} - \text{Fecal Loss}}{\text{Food intake}} \text{ X } 100$$

Indigestible gut fill (Vn) was calculated as: Vn = F * MRT, where F is daily output of feces. Total gut fill (V) was calculated as: V = [V – Vn/(1-D)]/[ln(1-D)], where D is the fractional digestibility. This assumes that net absorption is exponential with time (Holleman and White 1989; Barboza 1995). For analysis among seals, gut fill data were standardized to body mass ($Kg^{1.0}$, Barboza and Bowyer 2000; Demment and Van Soest 1985).

Repeated measures of food intake, digesta passage, gut fill and digestive efficiency were compared for effects of season, feeding frequency and diet by analysis of variance with body mass as a covariate (MANCOVA). Proportions or percentages were transformed to the arcsine of the square root before analysis (Zar 1999). Least squares linear regressions were used to assess bivariate relationships among average mass during each period, intake, MRT, and digestibility for each feeding trial. Statistical significance was set at $\alpha = 0.05$. Means are reported as mean $\pm$ 1 SE. MRT and intake were standardized to body mass for graphical presentation ($Kg^{0.75}$; Martin et al. 1985)

Results

Body mass of captive harbor seals fluctuated throughout the six feeding trials at the ASLC. Although one seal, Cecil, had a higher overall mean mass when fed herring (Pollock, 73.7 $\pm$ 4.0; Herring, 85.5 $\pm$ 3.2, $P < 0.05$, Table 5.1), body mass did not differ with diet for all other animals.

Marker recovery and retention time

Markers were excreted as a symmetrical pulse for both particulate and solid phases of digesta (Figure 5.1, $P > 0.05$). Pairwise comparison of t50 between solid and liquid markers indicated phase separation during high feeding frequency ($P < 0.05$) but not during low feeding frequency. Phase separation at low feeding frequency may be underestimated by absorption of Co from the digestive tract.

Fecal recoveries of Cr and Co doses after 72 h were 81.7% (± 12.3%) and 53.3% (±13.4%), respectively. Recovery of Co at low FF was only 35% ± 17.5% whereas Co recoveries on high FF was 71% (± 9.5%) and similar to that of the solid marker. It is likely that an unknown amount of the liquid marker was absorbed and eliminated in the urine as reported in studies of herbivores (Van Soest et al. 1998). Recovery of the solid marker is similar to those reported for other studies of pinnipeds (Goodman-Lowe et al. 1997).

Mean retention times of markers were variable and similar between diets and feeding frequencies (Figure 2, $P > 0.05$). The lowest measure of MRT was 11.2h for pollock at high FF whereas the longest MRT was 29.4 h for herring at low FF. Gut fill was estimated on the basis of dry matter in feces, which ranged from 17% to 61% (N = 594). Estimates of digesta dry mass in the gut were lower for herring than for pollock (1 vs 2% of body mass; Figure 5.3, $P < 0.005$). High feeding frequency also increased gut fill on pollock (low FF, x = 1.4%; high FF, x = 2.1%, $P < 0.05$).

Diet analysis

Herring contained more lipid and ash (48.9 ± 1.8% lipid of DM; ash, 3.5 ± 1.4% ash of DM) than pollock (lipid, x = 21.9% ± 1.7%; ash, x = 1.4% ± 0.3%; $P < 0.001$, N = 80). Pollock contained more protein than herring (Pollock, crude protein, x = 77.1% ± 4.3%; Herring, x = 4.9% ± 2.0%, $P < 0.001$). The mixed diet contained 72.1% (±

2.1%) water and a mean estimated DM crude protein value of 52.7% (± 3.4%). Proximate composition of fish species fed to harbor seals did not change over the course of this experiment.

Intake and digestibility

Body mass, intake, and digestive efficiency were similar between seasons ($P > 0.05$). Feeding frequency did not influence overall intake within each diet, however. Intake of pollock was 30% higher than that of herring (wwt intake, pollock, low FF, x = 4.1 ± 1.7 kg d^{-1}, high FF 4.7 ± 2.0 kg d^{-1}; herring, low FF, x = 1.9 ± 0.6 kg d^{-1}, high FF, x = 2.0 + 0.5 kg d^{-1}; $P > 0.05$). This equated to a mean dry matter intake of 0.92 ± 0.35 kg d^{-1} for pollock and 0.64 ± 0.23 kg d^{-1} for herring. Pollock intake was 10% greater than intake of the mixed prey diet (0.83 ± 0.39 kg.d^{-1}, $P < 0.05$). Protein intake was greatest from pollock (wwt; pollock, x = 2483.8 ± 179.8 g d^{-1}; herring, x = 1081.3 ± 72.1 g d^{-1}; mixed, x = 1603.4 ± 170.4 g d^{-1}; $P < 0.001$) and increased with feeding frequency (x = 2946.4 ± 421.1 g d^{-1}; x = 1416.5 ± 281.4 g d^{-1}, $P < 0.05$). Lipid intake was greater on the herring (herring, x = 803.9 g d^{-1} ± 107.5; pollock, x = 573.3 ± 91.8 g d^{-1}; mixed, x = 408.7 ± 74.2 g d^{-1}, $P < 0.005$) but was not affected by feeding frequency (667.9 ± 125.0 g d^{-1} vs. 522.3 ± 91.4 g d^{-1}; $P > 0.05$). Dry matter digestibility did not change with season, feeding frequency or diet (90.3% ± 2.9%, Figure 5.4A).

Digestibility of protein and lipid differed with diet. Protein digestibility was lower for herring (65.2 $\pm$ 1.9%) than for either pollock (81.1 $\pm$ 1.4%) or the mixed diet (78.4 $\pm$ 1.6%, $P < 0.005$, Figure 5.4B). High feeding frequency of herring reduced lipid digestibility below all other diets and feeding frequencies (Figure 5.4C). While lipid digestibility for herring and pollock were similar at low FF, lipid digestibility declined with lipid intake when seals were fed herring at high FF (least squares linear regression, $P < 0.05$, Figure 5A). Intake of lipid above 60 g DM $Kg^{-0.75}$ d^{-1} was associated with digestibilities of only 50%. Protein digestibility was maintained across a wide range of intakes and exceeded the highest load of lipid by a factor of 3 (Figure 5.5B).

Feeding frequency did not influence DEI (pollock, low FF, x = 22521.5 $\pm$ 6239.6 kJ d^{-1}, high FF, x = 22838.6 $\pm$ 4709.1 kJ d^{-1}; herring, low FF, x = 14920.7 $\pm$ 5267.1 kJ d^{-1}, high FF, x = 15942.2 $\pm$ 7170.0 kJ d^{-1}; $P > 0.05$). Low frequency feeding on pollock provided DEI values (1620.6 $\pm$ 223.8 kJ d^{-1}) that were 26% greater than for herring and 22% greater than that of mixed diets (herring, x = 1191.8$\pm$ 164.2 kJ d^{-1}; mixed, x = 1256.0 $\pm$ 205.8 kJ d^{-1}; $P < 0.01$, Figure 5.6). Dietary differences in DEI were diminished at high feeding frequency (pollock, 1752.8 $\pm$ 230.9, herring, 1517.8 $\pm$ 257.0, mixed 1457.8 $\pm$ 212.9; $P > 0.05$, Figure 5.6).

Discussion

Increasing the load of DM entering the digestive tract of captive harbor seals did not reduce digestibility of DM. Consequently gut fill increased with the frequency of consuming the high protein prey (pollock). Our data do not support the hypothesis that feeding frequency alters total retention in the digestive tract but do indicate that feeding frequency alters segmental flow.

Seals have simple digestive tracts dominated by long tubular intestines (Helm 1983, Stevens and Hume 1997). Consequently, both fluid and solid markers were excreted as a symmetrical pulse. Therefore, mixing of digesta may be achieved by segmental contractions of the intestine rather than by a single compartment such as the stomach. The separation of marker phases at high feeding frequency is consistent with relatively incomplete mixing of digesta contents. In the only other study to use both solid and liquid phase markers to measure particle flow in pinnipeds, Krockenberger and Bryden (1994), described a similar pattern of phase separation in elephant seals. Similar mean retention times between rates of intakes among seals in this study may simply reflect differences in the contribution of gastric and intestinal delays, that is, retention in the stomach may be more important when large meals are consumed, such as in the high gut fill for pollock (Fig. 5.2).

During this study we witnessed longest MRT values when seals were fed herring. MRT for harbor seals fed herring for both the solid and liquid phase markers ranged

from 18.4 h to 29.4 h (low FF) to 15.4 h to 27.1 h (high FF). Pollock revealed lower values for both feeding frequencies, and ranged from 12 h to 24.9 h (low FF) to 9.2 h to 22.7 h (high FF). This range of retention is consistent with a reduction in gastric emptying and intestinal motility when dietary lipid is increased (Forbes and Swift 1944; Stevens and Hume 1995). However, our use of liquid and solid phase digesta markers over the entire digestive tract does not provide the resolution of segmental flow to characterize gastric and intestinal responses to lipid and protein load. While few studies have reported solid and liquid phase retention times in phocids, our data corresponds to previous results reported for other pinnipeds such as elephant seals. Krockenberger and Bryden (1993) also used a liquid and a solid phase marker and calculated MRT's of 13 h for juvenile elephant seals. Our data are similar to those for terrestrial carnivores such as domestic dogs, cats and raccoons, which have retention times of 22, 13 and 15 h respectively (Warner 1981).

Our results for captive harbor seals are consistent with previously reported dry matter digestibilities for pinnipeds. This study estimated DMD values averaging 92% for herring and 88.5 for pollock. Fadely et al. (1994) reported that California sea lions had DMD ranging from 88% to 91% for herring and 83% to 91% for pollock. Rosen and Trites (2000a) found lower DMD for pollock (86.5%) and for herring (90%) in captive Steller sea lions using manganese as an indigestible marker. The small difference between these measures may reflect differences in diet composition, species of pinniped and the use of manganese (Mn) as a marker (Barboza and Jorde 2001).

An increase in the feeding frequency of pollock increased gut fill in captive harbor seals to a maximum intake (Figure 3) of 10% of body mass (if digesta moisture is assumed to be 80%). This is similar to the gut capacity of several mammals (Stevens and Hume 1995), and suggests that increased food abundance may stimulate hyperphagia in seals even though demands for energy and nutrients are not altered. This observation is consistent with a flexible foraging strategy that utilizes patchily distributed prey but also employs a flexible gut fill. Ad libitum intakes of herring in captive harbor seals during this study (400 - 2000 g d^{-1}) encompassed the range observed in other studies with this prey (541 g d^{-1}; Ashwell-Erickson and Elsner 1981). Feeding frequencies may therefore influence responses to diets in captive pinnipeds. For example, low feeding frequency of herring and pollock for captive Steller sea lions, resulted in losses of body mass on pollock but not on herring (Rosen and Trites 2000b). Disparate responses of sea lions and seals to this same prey may also reflect effects of season and different digestive responses to protein and lipid load.

We found that food intake of harbor seals increased when fed a relatively low-energy diet of pollock compared with herring at low FF. In a study involving rats, it has been suggested that there is a positive relationship between food intake and lipid levels (Burton-Freeman et al. 1999). This compensatory response of increased intake for reduced energy density in the diet is consistent with other studies of small mammals such as rats (Donnelly et al. 2003) and shrews (Woodall and Currie 1989). This is the

first study to demonstrate the compensatory response in pinnipeds. Previous studies of Steller sea lions and harp seals fed ad libitum did not find an increase in food intake when diets were switched from herring to pollock. Consequently, those animals lost body mass (Rosen and Trites 1997) and fat reserves (Kirsch et al. 2000).

Our hypothesis of a trade-off between intake and digestion was not supported for protein but was supported for lipid. Although no difference was witnessed in overall intake between feeding frequencies, seals fed herring at high FF often exhibited steatorrhea (oily feces), which suggests that the ability to remove dietary lipids from the gut was exceeded. Similar loads of lipid between 40 and 60 gDM $kg^{-0.75}$ d^{-1} from pollock and mixed diets did not reduce lipid digestibility in the same animals (Figure 5A). Therefore, lipid and protein from herring may be more difficult to digest than those from pollock. Low protein digestibility in herring at both feeding frequencies may reflect a combination of high concentrations of structural proteins as well as the effect of lipid rendering dietary protein less accessible to proteolytic secretions. Dietary loads of lipid above 60 gDM $Kg^{-0.75}$ were mainly from herring and thus the reduced digestibility of lipid was probably due to one or more of the following steps: emulsification with bile, lipolysis from pancreatic lipases, absorption of micelles from the gut or limits to clearance of chylomicrons from the enterocytes (Hamosh et al. 1994). Unknown differences in structure of lipids between prey species may also contribute to the differences in lipid digestion but that suggestion awaits conformation from studies of lipid composition, uptake and flow.

Poor responses to lipid loads from herring were probably not due to inadequate acclimatization of digestive systems in captive harbor seals. Most studies involving digestive efficiencies or retention time have acclimation periods ranging from 5 to 21 days (Ashwell-Erickson and Elsner 1981, Rosen and Trites 2000b). The lower end of these acclimation periods may not provide sufficient time for enzymatic modulation, especially after switching from prey differing in composition (Hilton et al. 2000). In order to circumvent potential digestion/enzymatic constraints, we employed an acclimation period of approximately 90 days prior to caging animals, which is generally adequate for inducing pancreatic and intestinal acclimation of mammals and birds (Karasov and Hume 1997). It is possible that previous studies of captive pinnipeds did not allow a long enough acclimation period to override problems of palatability or other behaviors. Harbor seals during this study were fed to maximum voluntary intake at levels ranging from 1.5 to 8 times maintenance energy intake (based on a predicted value of 70 kcal x $kg^{0.75}$ x day^{-1}). The apparent consistency of DM digestibility across intakes may be typical to vertebrate prey and is similar to that observed for harp seals (Phoca groenlandica) fed herring at 0.5 to 1.3 times predicted maintenance intake (Keiver et al. (1984). During this study harbor seals on high FF of pollock increased DM intake by 42% and DEI by 13% over those parameters for herring at the same feeding frequency. For harbor seals, this equates to approximately a 30% increase in pollock to equal the energy intake of herring. It has been reported that young sea lions would require upwards of 80% more pollock than herring to

maintain similar energy intakes (Rosen and Trites 2000b). Digestible intake of energy in captive seals was similar to field metabolic rates for pinnipeds (Nagy et al. 1999; Reilly and Fedak 1991). Therefore the digestive performance of animals in our study probably approximates that of free-living animals. Although we found no effects of season, it is possible that seasonal reproduction, molt and mass gain of wild animals could alter digestive morphology as well as clearance of absorbed nutrients.

Our data suggests that DEI of captive harbor seals exceeded their energy requirements without altering body mass. High intakes of excess energy may result in diet-induced thermogenesis (DIT). We did not measure heat production in these captive harbor seals but several sources suggest that energy retention in body tissue may be altered by DIT or by shifts in substrate oxidation (Feist and White 1989; Markussen et al. 1994; Rosen and Trites 1997; Dulloo and Samec 2001). For example, felines can maintain body weight during short-term isocaloric feeding of a high-fat meat diet by increasing fat oxidation commensurate with increases in fat intake (Lester et al. 1999). This ability may be an important mechanism underlying the resistance to weight gain, despite habitual consumption of high-energy diets in pinnipeds.

In the face of foraging in a patchy environment, intake of a more energy dense prey item would seem advantageous, since it would maximize the rate of acquisition of metabolizable energy, could lower the heat increment of feeding (Markussen et al. 1994) and thus minimize the cost of foraging. However, results of this study imply that

a flexible digestive system for harbor seals can compensate for ingesting a prey of low energy density by increasing gut fill and enhancing protein and lipid assimilation, to sustain digestible energy intake. In other words, harbor seals can offset differences in prey quality if limits on prey availability and abundance do not exceed the physiological plasticity of their digestive system to maintain their supply of energy and nutrients.

Acknowledgements

We would like to thank the support staff from the Alaska SeaLife Center. This work was conducted under Marine Mammal Protection Act # 881-1443 to M.A.C and the ASLC, University of Alaska IACUC (97-28) and ASLC IACUC (99-01) permits for the resident harbor seals. We would like to acknowledge The Elmer Rasmuson Fisheries Fellowship for support throughout this project. We would also like to thank the following for their assistance with this project; M. Bando, J. Castellini, L. Cornick, H. Harmon, S. Harper, T. Mau, L. Petrauskas and S. Schexnailder. The research described in this paper (Restoration Project 01341) was supported by the Exxon Valdez Oil Spill Trustee Council. However, the findings and conclusions presented by the authors are their own and do not necessarily reflect the views or position of the Trustee Council.

Literature cited

Anthony JA, Roby DD, Turco KR (2000) Lipid content and energy density of forage fishes from the northern Gulf of Alaska. JEMBE 248: 53-78

Ashwell-Erickson S, Elsner R (1981) The energy cost of free existence for Bering Sea harbor and spotted seals. In: Hood, D.W. and J.A. Calder (eds.), The Bering Sea Shelf: Oceanography and Resources, Vol. 2., pp 869-899. Univ Wash Press

Barboza PS (1995) Digesta passage and functional anatomy of the digestive tract in the desert tortoise (Xerobates agassizii). J. Comp Physiol B 165: 193-202

Barboza PS and DG Jorde. 2001. Intermittent feeding in a migratory omnivore: digestion and metabolism of American Black Duck during autumn. Physiol Biochem Zool 74:307-317

Barboza PS, Bowyer RT (2000) Sexual segregation in dimorphic deer: a new gastrocentric hypothesis. J Mammal 81:473-489

Blaxter KL (1989) Energy metabolism in farm animals. Cambridge Univ Press, Cambridge UK

Biswas S, and Sankar K (2002) Prey abundance and food habit of tigers (Panthera tigris tigris) in Pench National Park, Madhya Pradesh, India. J Zool 256:411-420

Brown RF, Mate BR (1983) Abundance, movements, and feeding habits of harbor seals, Phoca vitulina , at Netarts and Tillamook Bays, Oregon. Fish Bull. 81: 291-301

Burton-Freeman B, Gietzen DW, Schneeman BO (1999) Cholescystokinen and serotonin receptors in the regulation of fat-induced satiety in rats. Am J Phys 276:R429-R434

Calkins DG, Becker EF, Pitcher KW (1998) Reduced body size of female Steller sea lions from a declining population in the Gulf of Alaska. Mar Mamm Sci 14 (2): 232-244

Caro TM (1994) Cheetahs of the Serengeti plains. Chicago: The University of Chicago Press.

Carbone C, Mace GM, Roberts SC, Macdonald DW (1999) Energetic constraints on the diet of terrestrial carnivores Nature 402:286-288

Castellini MA, Castellini JM, Trumble SJ (2002) Recovery of harbor seals. Phase II: Controlled studies of health and diet. Exxon Valdez Oil Spill restoration Project Annual Report. 40 pp.

Davis DD (1962) Allometric relationship in lions vs. domestic cats. Evol 16: 28-29

Demment MW, Van Soest PJ (1985) A nutritional explanation for body-size patterns of ruminant and non-ruminant herbivores. Am Nat 125: 641-672

Donnelly CP, Trites AW, Kitts DD (2003) Possible effects of pollock and herring on the growth and reproductive success of Steller sea lions (Eumetopias jubatus): insights form feeding experiments using an alternative animal model, Rattus norvegicus. Brit J Nut 89:71-82

Dulloo AG, Samec S (2001) Uncoupling proteins: their roles in adaptive thermogenesis and substrate metabolism reconsidered. Brit J Nutr 86: 123-139

Fadely BS, Worthy GAJ, Costa DP (1990) Assimilation efficiency of northern fur seals determined using dietary manganese. J Wildl Manag 54:246-251

Feist DD, White RG (1989) Terrestrial mammals in cold. In Advances in comparative and environmental physiology. Vol. 4. Edited by L. C. H. Wang. Berlin: Springer-Verlag

Fisher KI, Stewart REA, Kastelein RA, Campbell LD (1992) Apparent digestive efficiency in walruses (Odobenus rosmarus) fed herring (Clupea harengus) and clams (Spisula sp.). Can J Zool 70: 30-36

Forbes EB, Swift RW (1944) Associative dynamic effect of protein, carbohydrate and fat. J Nutr 27:453-468

Goodman-Lowe GD, Atkinson S, Carpenter JR (1997) Initial defecation time and rate of passage of digesta in Hawaiian monk seals, Monachus schauinslandi. Can J Zool 75: 433-438

Hamosh M, Iverson SJ, Kirk CK, Hamosh P (1994) Milk lipids and neonatal fat digestion: relationship between fatty acid composition, endogenous and exogenous digestive enzymes and digestion of milk fat. World Rev Nutr Diet 75: 86-91

Helm RC (1983) Intestinal length of three California pinniped species. J Zool Lond 199:297-304

Hilton GM, Furness, RW, Houston DC (2000) The effects of diet switching and mixing on digestion in seabirds. Func Ecol 14: 145-154

Holleman DF, White RG (1989) Determination of digesta fill and passage rate from nonabsorbed particulate phase markers using the single dosing method. Can J Zool 67: 488-494

Jumars PA, Martinez del Rio C (1999) The tau of continuous feeding on simple foods. Physiol Biochem Zool 72:633-641

Karasov WR, Hume ID (1997) Vertebrate gastrointestinal system. In: Dantzler WH (ed) Handbook of Physiology, Section 13, Comparative Physiology. Volume I, Oxford University Press, pp 409-480

Kastelein RA, Vaughan N, Wiepkema PR (1990). The food consumption of Steller sea lions (Eumetopias jubatus). Aquat Mammals 15(4):137-144

Keiver KM., Ronald K, Beamish FWH (1984) Metabolizable energy requirements for maintenance and faecal and urinary losses of juvenile harp seals (Phoca groenlandica). Can J Zool 62: 1751-1756

Krockenberger MB, Bryden MM (1994) Rate of passage of digesta through the alimentary tract of southern elephant seals (Mironga leonina)(Carnivora: Phocidea). J Zool London 234: 229-237

Lawson JW, Hare JA, Noseworthy E, Friel JK (1997) Assimilation efficiency of captive ringed seals (Phoca hispida) fed different diets. Polar Biol 18: 107-111

Lester T, Czarnecki-Maulden G, Lewis D (1999) Cats increase fatty acid oxidation when isocalorically fed meat-based diets with increasing fat content. Am J Phys-Reg Integ Comp Phys 277 (3): R878-R886

Markussen NH, Ryg M, Oritsland NA (1994) The effect of feeding on the metabolic rate in harbour seals (Phoca vtiulina). J Comp Physiol 164: 89-93

Martin RD, Chivers DJ, Maclarnon AM, Hladdik CM (1985) Gastrointestinal allometry in primates and other mammals. pp. 61-90 in Jungers WL (ed.), Size and scaling in primate biology. Plenum Press, New York.

Nagy KA, Girard, IA, Brown TK (1999) Energetics of free-ranging mammals, reptiles, and birds. Annu Rev Nutr 19: 247-277

Paltridge D. (2002) The diets of cats, foxes and dingoes in relation to prey availability in the Tanami Desert, Northern Territory. Wildl Res 29(4):389-403

Penry DL, Jumars PA (1987) Modeling animal guts as chemical reactors. Am Nat 129: 69-96

Perrin MR (1994) Herbivory and niche partitioning. Pp. 128-149 in: The digestive system in mammals: food, form, and function (DJ Chivers and P Langer, eds.). Cambridge University Press, Cambridge, United Kingdom

Reilly JJ, Fedak MA (1991) Rates of water turnover and energy expenditure of free-living males common seals (Phoca vitulina). J Zool 223: 461-468

Robbins CT (1993) Wildlife nutrition and feeding. Academic Press, San Diego, 2nd edition. 352 pp

Rosen DAS, Trites AW (1997) Heat increment of feeding in Steller sea lions, Eumetopias jubatus. Comp Bio Phys 118A:877-881

Rosen DAS, Trites AW (2000a) Digestive efficiency and dry-matter digestibility in Steller sea lions fed herring, pollock, squid, and salmon. Can J Zool 78:234-239

Rosen DAS, Trites AW (2000b) Pollock and the decline of Steller sea lions: testing the junk-food hypothesis. Can J Zool 87: 1243-1250

Shipley LA, Gross JE, Spalinger DE, Hobbs NT, Wunder B (1994) The scaling of intake rate in mammalian herbivores. Am Nat 143: 1055-1082

Sibly RM, Calow P (1986) Physiological ecology of animals: an evolutionary approach. Blackwell, Oxford, UK.

Sidwell VD, Foncannon PR, Moore NS, Bonnet JC (1974) Composition of the edible portion of raw (fresh or frozen) crustaceans, finfish and mollusks. I. Protein, fat, moisture, ash, carbohydrate, energy value, and cholesterol. Mar Fish Rev 36: 21-35

Stevens CE, Hume ID (1995) Comparative physiology of the vertebrate digestive system. Cambridge Univ. Press. 2nd ed. 398 pp.

Uden P, Colucci PE, Van Soest PJ (1980) Investigations of chromium, cerium and cobalt as markers in digesta rates of passage studies. J Sci Food Agric 31: 625-632

Warner ACI (1981) Rate of passage of digesta through the gut of mammals and birds. Nutr Abstr Rev 51: 789-820

Williams TM, Haun J, Davis RW, Fuiman LA, Kohin S (2001) A killer appetite: metabolic consequences of carnivory in marine mammals. Comp Biochem Physiol Mole Integr Physiol 129 (4): 785-796

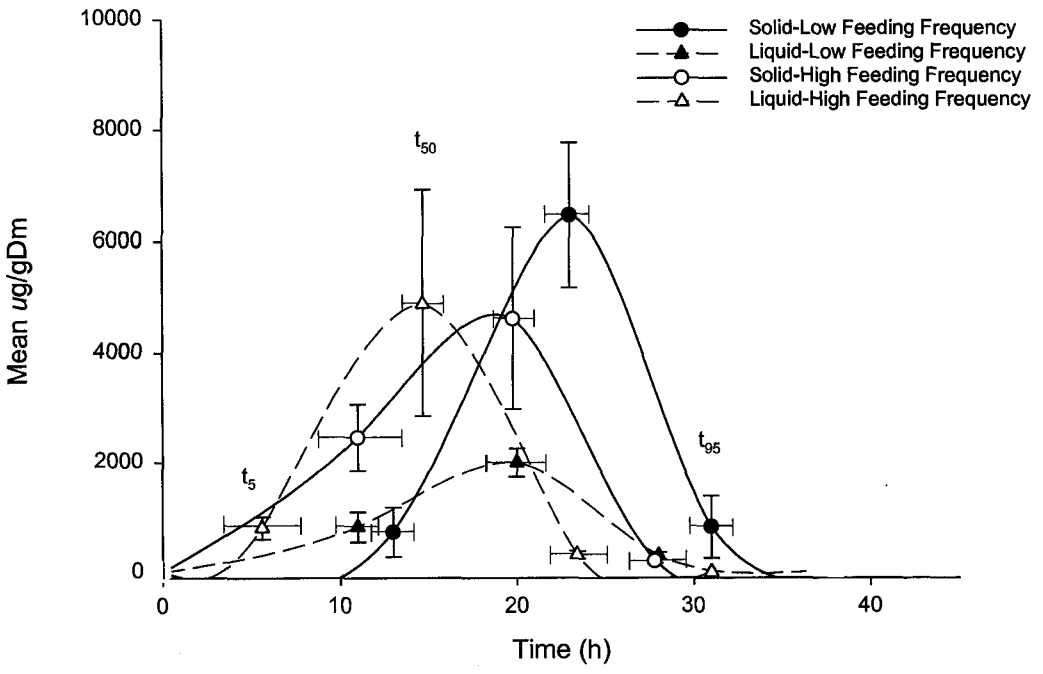

Figure 5.1. Concentration of markers in feces following a single oral dose of Cr_2O_3 and Co–EDTA to harbor seals (n = 8) fed at high (4X d^{-1}) (open symbols) or low (1X d^{-1}) (closed symbols) feeding frequencies. Serial symbols on each line represent times of excretion at 5% (t_5), 50% (t_{50}) and 95% (t_{95}) of the dose recovered in feces. Error bars are $\pm$ SE

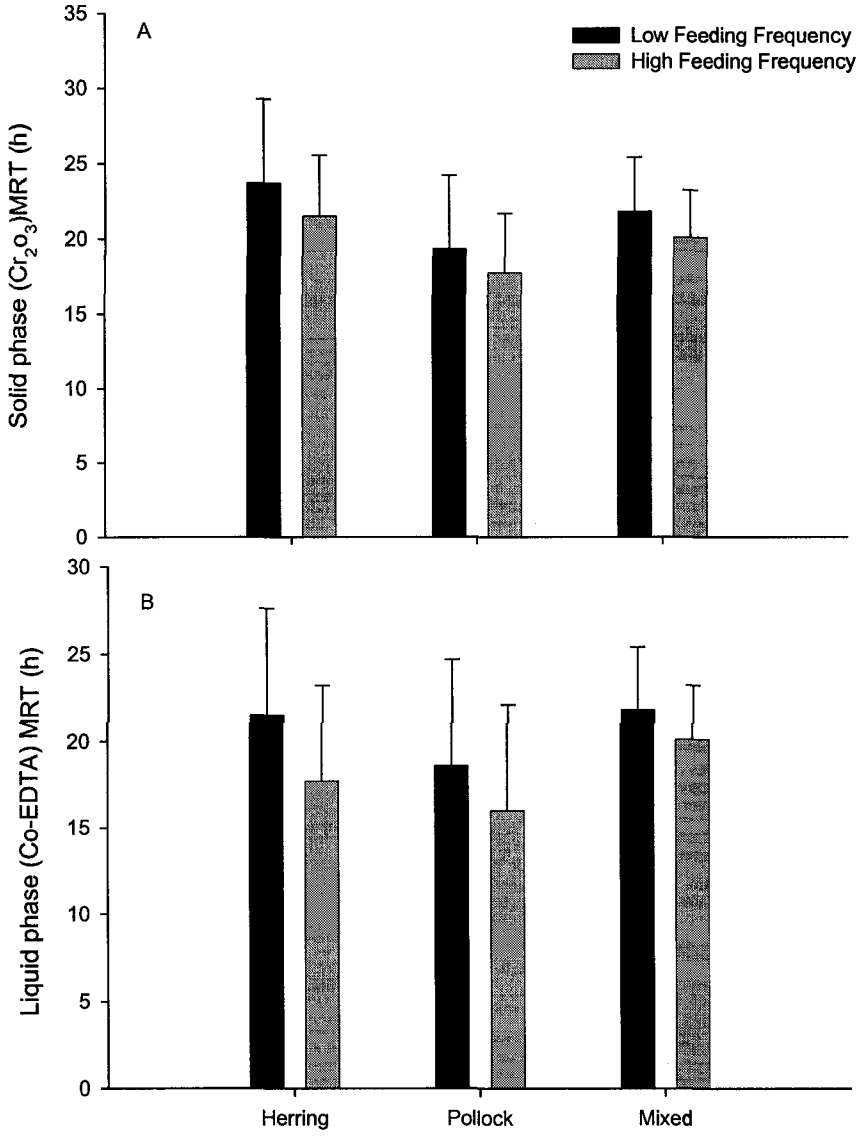

Figure 5.2. Mean retention time of solid phase (A; Cr_2O_3) and liquid phase (B; Co-EDTA) markers in captive harbor seals fed at high (4X d^{-1}) and low (1X d^{-1}) feeding frequencies on a diet of herring, pollock or an equal mixture of both species. Error bars are $\pm$ SE

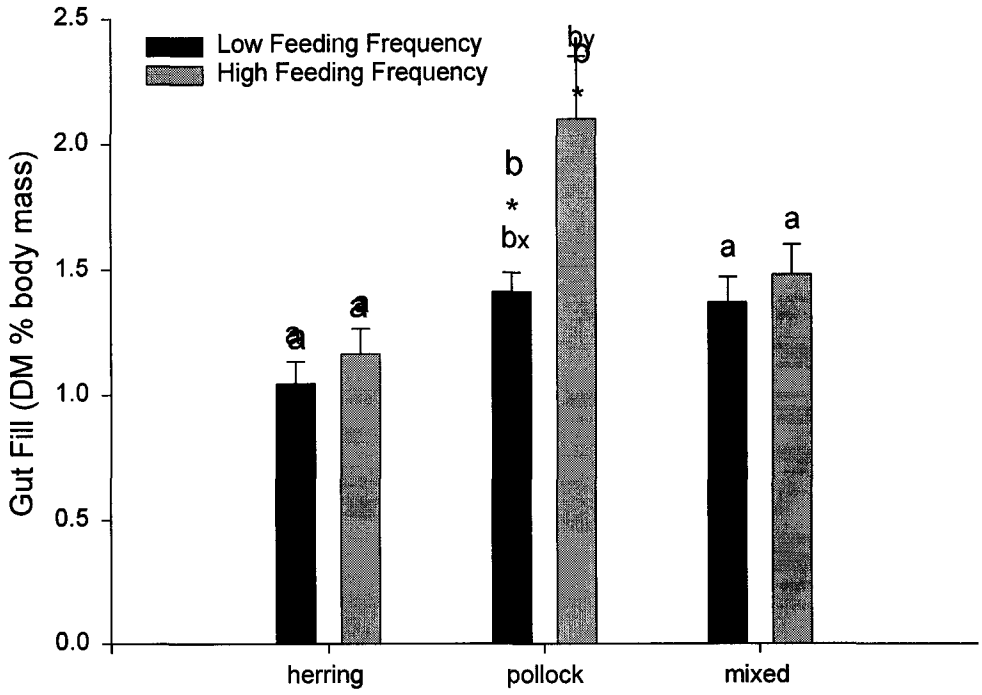

Figure 5.3. Estimated content of dry matter of the digestive tracts of harbor seals fed at high (4X d^{-1}) and low (1X d^{-1}) feeding frequencies on a diet of herring, pollock or an equal mixture of both species. Dissimilar letters indicate statistical differences whereas * indicates differences within prey feeding frequency. Error bars are + SE

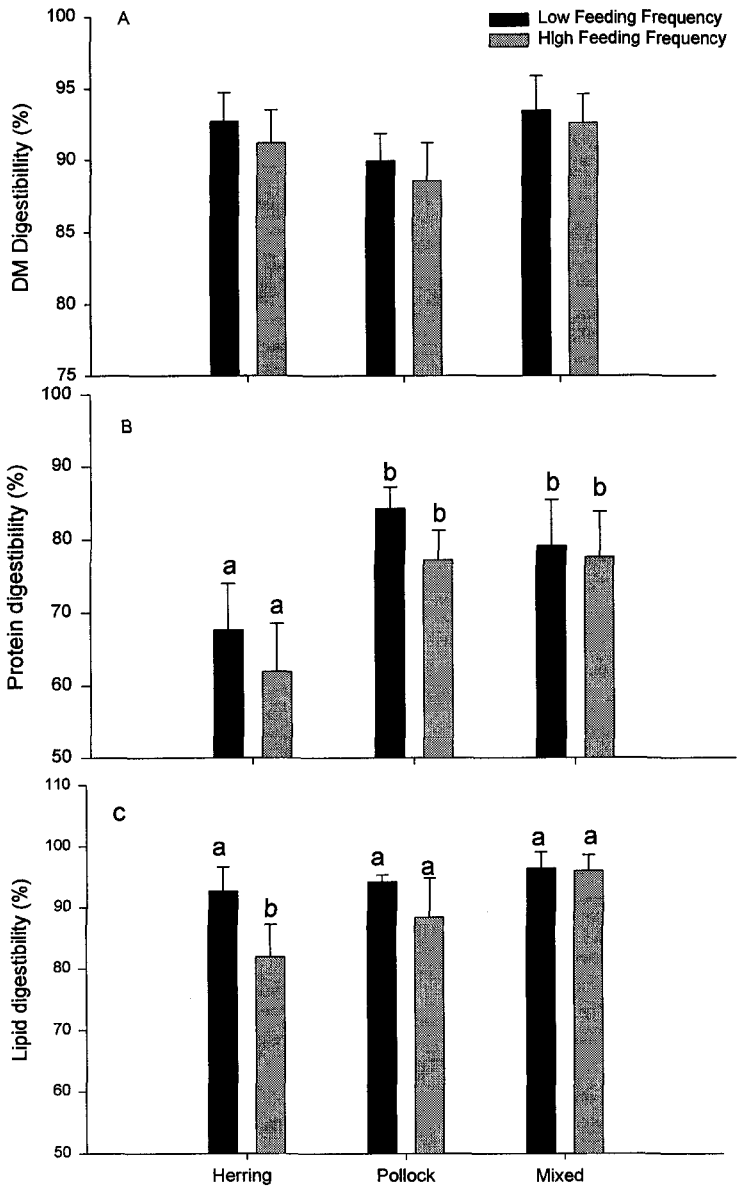

Figure 5.4. Digestibilities of dry matter (4A), protein (4B) and lipid (4C) in harbor seals fed at high (4X d^{-1}) and low (1X d^{-1}) feeding frequencies on a diet of herring, pollock or an equal mixture of both species. Dissimilar letters indicate statistical differences. Error bars are + SE

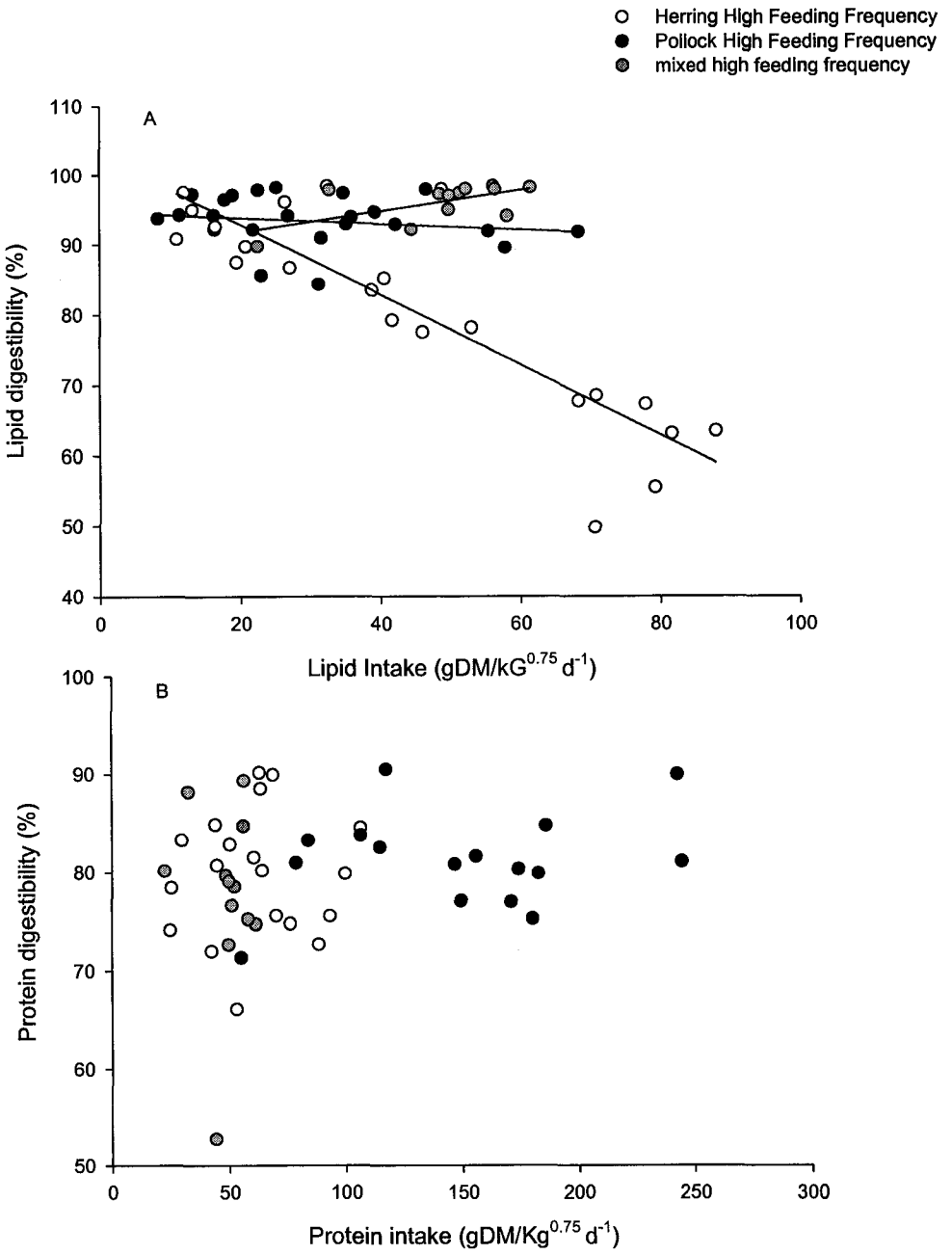

Figure 5.5. Relationships between intake and digestibility of lipid (A) and protein (B) for harbor seals fed at high feeding frequency (4X d^{-1}), herring, pollock or an equal mix of both species. Solid lines indicate significant ($P<0.05$) least squares linear

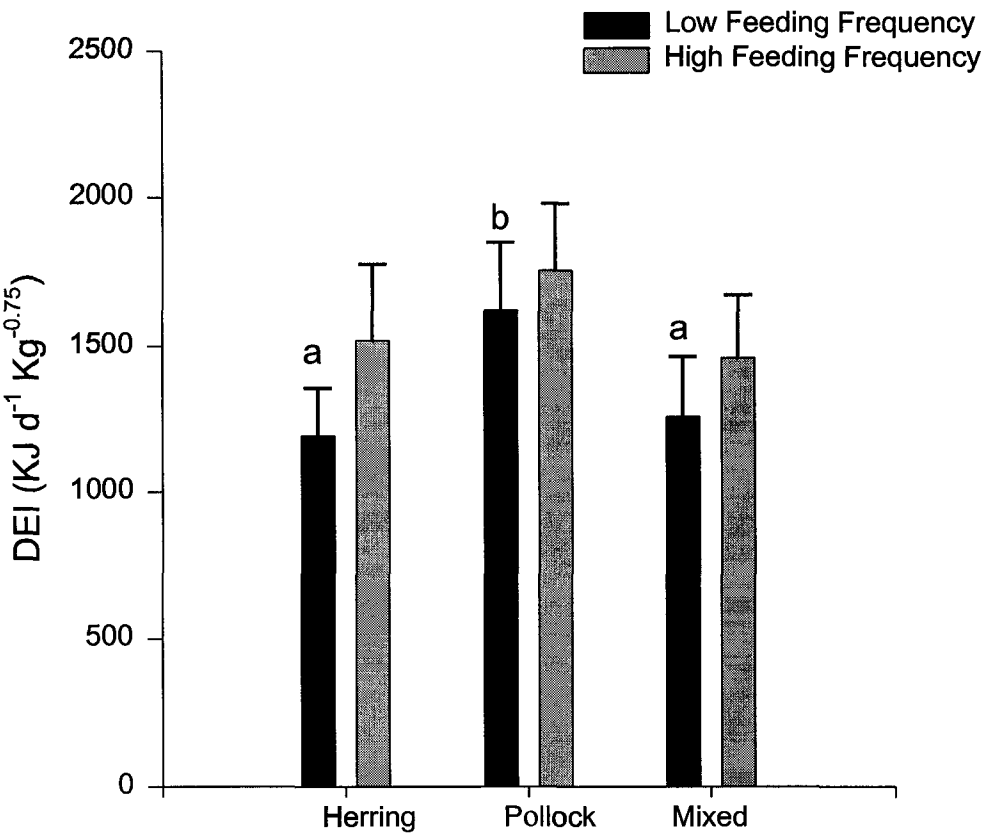

Figure 5.6. Digestible energy intake (DEI kJ d^{-1} $kg^{0.75}$) for harbor seals fed at high (4X d^{-1}) and low (1X d^{-1}) feeding frequencies on a diet of herring, pollock or an equal mixture of both species. Dissimilar letters indicate statistical differences ($P<0.05$). Error bars are $\pm$ SE

Table 5.1. Mean masses during caged feeding trials for 8 captive harbor seals at the ASLC during six feeding trials (FT). Seals were fed herring (h) or pollock (p) or a equal herring/pollock mixed diet (Mixed).

Seal ID	Age	Sex	Feeding regime	FT1 (SE)	FT2 (SE)	FT3 (SE)	FT4 (SE)	FT5 (SE)	FT6 (SE)
Cecil	15	M	p/h/p/h/p/h	80.1 (2.7)	91.8 (0.3)	68.6 (3.2)	75.8 (1.3)	72.3 (1.8)	88.9 (3.1)
Skeezix	23	F	p/h/p/h/p/h	66.5 (0.8)	67.5 (0.8)	67.0 (1.5)	80.8 (2.4)	65.3 (1.0)	68.3 (2.2)
Poco	23	F	p/h/p/h/p/h	59.5 (1.0)	57.0 (1.6)	59.0 (1.0)	72.6 (4.0)	59.5 (1.2)	63.0 (4.1)
Travis	3	M	h/p/h/p/h/p	38.3 (2.6)	33.3 (0.5)	47.5 (1.3)	37.9 (2.5)	36.0 (2.5)	37.5 (2.4)
Pender	3	M	h/p/h/p/h/p	42.6 (2.0)	39.2 (0.8)	47.8 (1.5)		47.3 (1.1)	46.0 (2.1)
Sydney	3	F	h/p/h/p/h/p	40.0 (0.5)	34.8 (0.3)	51.6 (1.4)	36.9 (1.7)	44.3 (1.8)	50.3 (2.0)
Snapper	15	M	Mixed h/p	84.9 (3.3)	81.7 (0.6)	86.1 (1.9)	76.9 (2.5)	86.9 (1.7)	82.8 (4.3)
Tina	7	F	Mixed h/p	56.5 (3.0)	52.6 (0.9)	67.0 (2.9)	59.9 (0.9)	54.8 (1.5)	70.8 (1.5)

6

CONCLUSION

The objective of this thesis was to address one of the currently most controversial topics in marine mammal ecology, the "junk-food" hypothesis. The marine ecosystem of Alaska experienced a "regime shift" in the mid 1970s that moved the system from a groundfish/herring-based food web to a pollock and arrowtooth flounder dominated food web. The high-energy food that pinnipeds and birds once consumed simply disappeared. The "junk-food" hypothesis was first proposed at a SeaGrant sponsored workshop in 1991 and subsequently published (Alverson 1992). This hypothesis stated that Alaskan waters had a sufficient biomass of pollock to support the pinniped and bird populations, but that pollock was nutritionally poor compared to other less common species, such as herring and capelin. Thus, the hypothesis proposes that seals and sea lions may be starving in a sea full of pollock.

The progression of the work in this dissertation enables better understanding of the physiology of harbor seal pups and adults in light of possible environmental stressors or dietary changes. Chapter 2 involves assessing whether blood chemistry values are different between harbor seal pup populations captured in an area of continued decline and an area were numbers are stable or slightly increasing. The

results of this particular study suggested that there were differences in blood chemistry, hematology and morphometric data from harbor seal pups from these two geographically distinct areas in Alaska. There were between-location differences in greater than half of all mean blood parameters measured, which translated into significant reference range differences between the stable and declining populations. I speculated that the differences in blood chemistry values might have been indicative of population health or a response to an environmental perturbation or change, such as prey quality. Because of the difficulty of linking these statistical differences and potential health perturbations to individual blood parameter differences (is one value high or the other value low?), I suggested establishing and using blood parameters as indices of temporal change (each population is its own control) especially the parameters influenced by external factors (i.e. nutrition). In other words, I warned against comparative health assessment by comparing so-called "normal" values from distinct populations of seals.

These data from Chapter 2 provided the idea to test whether or not populations were indeed different based on blood chemistry and also assess the health of individual seals and extrapolate to population health. It was obvious from Chapter 2 and previous studies that blood chemistry and hematological values appear to be sensitive to environmental variation. However, the previous studies most often compared blood chemistry values between individuals from different regions or from previously reported clinical reference ranges and interpreted health accordingly. Therefore there is

a need to understand population level differentiation in plasma chemistry values and thus assess the health of animals occupying the outlier regions of populations, since these regions are often associated with poor health. In Chapter 3, the objectives were threefold: 1. Describe an integrated suite of blood chemistry /hematology values that, as a group, can be used to define the metabolic identity of a population of animals. 2. Once a group of blood values were described, our goal was to define the blood values from individual animals that would be considered "outliers". 3. To use the metabolic identity and outlier theory to define population health of distinct groups of harbor seals.

For this research, data from two populations of harbor seal pups were combined with a population from California in order determine if it is possible to distinguish among populations using blood chemistry. Results revealed that harbor seal pups in each of the three groups tested were distinguished by blood chemistry values. I believe this is the first reported example of plasma chemistry values used as a discrimination technique among marine mammal populations, in order to describe the integrated metabolic translation of genetic makeup and how it responds to environmental input. The significance of this technique is that it uses parameters that are known to respond to environmental cues and health status. Thus, this presented a picture of how animals have translated their genetic makeup into a metabolic response. This would prove useful when interpreting health between populations. Since it was determined that blood chemistry values (or the singular decomposition of many blood parameters) are

population specific, it would be additionally useful to assess the status of outliers in the populations. I employed a 3-dimensional graphing technique to identify the individual outliers from each population. This proved to be an effective exploratory technique since it can reveal patterns that are easily obscured unless you look at the "cloud" of data points from an appropriate angle. Based on our plasma chemistry analysis of outliers, the pup populations in Alaska appeared to be equally affected by nutritional perturbations. It could not be determined whether this is an ecologically significant percentage of the population. However, this may prove to be a major tool in assessing long-term population health. For instance, a shifting in the metabolic identity in long-term blood collection studies can provide clues to changing environmental conditions. However, to understand if the environment influenced changes in blood chemistry parameters, we would have to understand IF and WHAT changes occur at all. This was not feasible in free-ranging populations. Therefore, we conducted a study involving captive harbor seals to determine if blood chemistry or hematology values changes with season or diet quality.

The primary objective of this work was to identify the physiological variations expressed as differences in blood biochemistry and hematology levels as they are influenced by seasons and food quality, and also to determine if changes in diet and/or season influence morphology.

Chapter 4 of this dissertation demonstrated significant physiological variations in blood biochemistry and hematology in captive harbor seals in response to a change

in food quality or season. As in terrestrial systems, captive marine mammals can provide valuable insight in to the relative contribution of external influences on specific physiological responses. To my knowledge, this represents the first study to focus on the possible combined influences of season and diet on blood chemistry, hematology and morphology in captive harbor seals over long time periods.

We detected a dietary influence in plasma concentrations of liver enzymes (ALT, AST and GGT) and the ratio of the blood values of BUN:creatinine, such that there were relative increases while on a pollock diet when compared to a herring diet. We also detected seasonal differences in the plasma concentrations of Na^+ and Cl^- in captive harbor seals, with increased levels found during September to December for both electrolytes and Na^+ levels also elevated during January to April. It was suggested that seasonal changes in physiology, for example during molt, were genetically influenced and may account for the differences in electrolyte levels.

It was also revealed that hematology values were influenced by season. We found peak Hct and Hb values during September to December with the nadir occurring during the period from May to August. Whether the drop in Hct/Hb is a function of the increased haul-out times or a physiological adaptation to molting is unknown at this time.

During this particular study we observed numerous seasonal variations in blood chemistry, which appear to be indicative of shifts in the seals metabolic physiology.

Although seasonal metabolic shifts are partially attributable to an endogenous rhythm, the intensity of their expression was most likely affected by nutritional changes. This begged the question; could digestive constraints ultimately affect seal metabolic physiology? It has been reported that Steller sea lions (Eumetopias jubatus) were unable to maintain mass on a diet consisting exclusively of walleye pollock (Theragra chalcogramma) when compared to a more lipid dense fish such as Pacific herring (Clupea pallasii) (Rosen and Trites 2000). This may suggest that responses to diets that are low in lipid may reflect low intakes or a constraint to their digestion and absorption. This became the impetus for Chapter 5 of this dissertation.

Aquatic carnivores must process large amounts of lipid and protein in each feeding bout. An intake of large meals requires large capacity in the foregut with a concomitant ability to produce digestive secretions and absorb the products of degradation (Stevens and Hume 1995). Although endogenous enzymes can degrade the substrates from vertebrate prey, net uptake from the digestive tract can be limited by demand for secretions and by the time required for digestion and absorption (Jumars and Martinez del Rio 1999; Penry and Jumars 1987). As a result, digestive efficiency may be reduced by high rates of digesta flow due to high rates of intake or low capacity of the digestive tract (Sibly and Calow 1986). This relationship between digestion and intake is of particular interest in declining populations of pinnipeds because prey type could theoretically limit the animals' ability to meet requirements for maintenance.

In Chapter 5, I examined changes in digestive efficiency that accompanied changes in nutrient loads in captive harbor seals. The hypothesis was that increased feeding frequency would increase nutrient loads on seals and thus reduce retention time and the digestive efficiency of natural prey.

Results indicated that increasing the load of dry matter entering the digestive tract of captive harbor seals did not reduce digestibility of dry matter. Consequently gut fill increased with the frequency of consuming the high protein prey (pollock). These data did not support the hypothesis that feeding frequency alters total retention in the digestive tract but do indicate that feeding frequency alters segmental flow. It was also shown that an increase in the feeding frequency increased daily intake of fish in captive harbor seals. This suggests that increased food abundance may stimulate hyperphagia in seals even though demands for energy and nutrients are not altered. In other words, seals consuming a low quality prey may have adapted by increasing feeding frequency to maintain adequate energy levels, and the increase in feeding frequency therefore induces hyperphagia making it possible for seals to increase intake over and beyond what is needed energetically. This could account for the fact that seals maintain weight during certain times even on a low fat diet. The hypothesis of a trade-off between intake and digestion was not supported for protein but was supported for lipid. Seals fed herring at high frequency often exhibited steatorrhea (oily feces), which suggested that the ability to remove dietary lipids from the gut was exceeded. It was shown that dietary loads of lipid above 60 gDM $Kg^{-0.75}$ reduced

digestibility of lipid and was probably due to one or more of the following: inadequate emulsification with bile, lipolysis from pancreatic lipases, absorption of the micelles from the gut or limits to clearance of chylomicrons from the enterocytes.

The tradeoffs and constraints witnessed in this study lead to differing interpretations. In the face of foraging in a patchy environment, intake of a more energy dense prey item would seem advantageous, since it would maximize the rate of acquisition of metabolizable energy, could lower the heat increment of feeding and thus minimize the cost of foraging. However, results of this study imply that ingesting a prey high in protein increases gut fill and enhances protein and lipid assimilation, to provide a greater digestible energy intake. Therefore, contrary to a recent study we propose that consuming pollock would not equate to eating "junk-food".

Collecting information on field and captive blood chemistry markers and their association with diet and season is essential to understanding population health and environmental impacts on the physiology of animals. The results from the various chapters throughout this dissertation indicate that linking field and captive blood chemistry markers may provide a greater understanding of the population health and also illustrate the complex nature of using blood chemistry to determine population health.

The blood chemistry differences shown between the two distinct pup populations (Chapter 2) included; Na, Cl, K, P, BUN, Creatinine, B:C, Protein,

Globulin, AP, and ALT. Interestingly, Na and Cl accounted for nearly 50% of the population variation in discriminating between populations (Chapter 3), with ALT, P, and Creatinine also included. Of the 7 variables found to account for 90% of the population discrimination, only albumin and cholesterol were not found to differ between the two populations.

From the captive study it was shown that Na, Cl and BUN were influenced by season. Although all samples from free-ranging harbor seals were collected during the same time period, the differences in these blood values may reflect the metabolic state of the animal that reflects both environmental stressors and the genetic capacity of the animal to respond to metabolic demands environmental pressures. The differences in blood chemistry values found between populations of free-ranging seals also included ALT, Creatinine and B:C, all of which were influenced by diet in the captive study. These may prove important blood chemistry markers for assessing dietary differences between populations of seals.

Future Research

The evidence set forth in this dissertation is not sufficient to conclude that the nutrition and environmental hypotheses given within these chapters are indeed fact. However, we now have a better understanding of methods to determine population health and digestive constraints in harbor seals, although it remains to be seen if this carries over to otariid and bird species also suffering population declines. In Chapter 2

there was evidence that the harbor seal pups in the declining population were heavier than in the population that has recently increased. While this may be a reflection of developmental differences (birth clines) between the two populations, it also may be a function of a reduced carrying capacity in the Gulf of Alaska and Kodiak area. The reduced carrying capacity argument states that the regime shift of 1976-77 altered the ecosystem such that preferred pinniped prey decreased or altered their distribution. It may or may not be coincidence, but the timing of these pinniped declines also matches the Pacific Decadal Oscillation and ecosystem regime shift. Linking climate variations to impacts on marine ecosystems is generally considered to be "direct", such that climate and associated environmental changes lead to changes in habitat suitability for a particular species or suite of species in an ecosystem. Examples of direct climate impacts on ecosystems include: rising temperatures that either cause thermal stress or exceed thermal tolerances for particular species; reduced sunlight due to increases in cloud cover that lead to reduced phytoplankton productivity; or increased current speeds that sweep larval fish away from nursery habitats in ways that reduce survival rates. To address these issues, an integrated study involving marine mammal researchers, ichthyologists, and oceanographers could assess the correlation between prey movements, sea temperatures and pinniped foraging behavior. While scientists may not have ideal measures of carrying capacity, there are measures that serve as proxies or indicators for carrying capacity. At the lower, unexploited, trophic levels such as plankton, measures of standing biomass are available. For fish, recruitment estimates are generally available going back centuries. For birds and marine mammals,

juvenile survival is the most commonly measured metric. Future studies should focus on basic ecological measures such as pup survival, juvenile survival after weaning, age a first reproduction, fecundity, birth mass, and gender differences in offspring between areas of decline and increase. This would entail long-term monitoring, since it appears that the carrying capacity for Alaska's marine mammals varies on decadal to interdecadal time scales. Using blood as a "metabolic identity" may provide evidence of shifts on these decadal time scales. At this time, evidence required to test these hypotheses from Alaska's marine waters is generally sparse and insufficient to arrive at definitive conclusions.